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## EMBRYOMAS IN PLANTS.
### (PRODUCED BY BACTERIAL INOCULATIONS.)


By Erwin F. Smith.

*(From the Laboratory of Plant Pathology, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.)*


In April, 1916, I announced the discovery of a new type of crown gall, *i. e.*, one containing numerous leafy shoots, and showed that I could produce it at will by making my bacterial inoculations in leaf axils where there is a dormant bud; that exceptionally in tobacco I had produced it on the blade of the leaf where there were no buds, also once in an internode; that frequently the secondary tumors were of the same type as the primary tumor, *i. e.*, full of perishable leafy shoots; and, finally, that I regarded it as a true embryoma comparable to those occurring in animals.[1] Since then I have been experimenting continuously and now wish to offer further data on the production of these anomalous crown galls which, following Adami's terminology of tumors (Principles of Pathology), I consider to be atypical teratoid tumors.[2] With one exception (Fig. 63), all the photographs and photomicrographs I shall use for illustration are of tumors which are the result of pure-culture bacterial inoculations, although I have since discovered that leafy crown galls occur in nature on various plants, *e. g.*, on the rose and on the carnation.

Atypical (that is, embryonal) teratoid tumors have now been produced by the writer in plants of the following genera: Pelargonium, Nicotiana (2 sp.), Lycopersicum, Citrus, Ricinus, Impatiens, Hibiscus, Allemanda, Mangifera, Opuntia, Fuchsia, Tropæolum, Coleus, Beta, Rosa, and Brassica (both cabbage and cauliflower), using pure cultures of the hop strain of the crown-gall organism (*Bacterium tumefaciens*), introduced by needle-pricks. These genera belong to 15 different families of plants. The writer has also produced them on the carnation (Dianthus) with a strain of the crown-gall organism cultivated from a leafy tumor on *Dianthus caryophyllus*, and on a third species of tobacco (*Nicotiana langsdorfii*) with a strain of the crown-gall organism (Resistant Daisy) cultivated from a tumor on the Paris daisy (*Chrysanthemum frutescens*). From this it is reasonable to suppose that embryonal teratoid tumors can be produced in a great variety of plants and as readily as the ordinary crown galls. All that is necessary is to introduce the crown-gall bacteria into the growing tissues of susceptible species in the vicinity of totipotent cells. These totipotent cells may be either dormant axillary buds or meristematic cells remote from leaf axils and buds, or bud anlage. These cells have the potentiality of germ cells whatever their origin, *i. e.*, somatic or germinal. It was to the production of embryomas on leaves and on stem internodes from ordinary meristem cells, presumably somatic cells, that a part of my energies were directed during the summer of 1916.

This paper treats principally of crown-gall teratoids produced on Nicotiana and Pelargonium, but photographs of a few other plants are introduced for specific purposes, *e. g.*, of

[1] The *Journal of Cancer Research*, April, 1916.

[2] This paper is an expansion of the latter part of an address given by permission of the Secretary of Agriculture before The Johns Hopkins Medical Society, December 18, 1916, entitled: "Is there any real relation between crown gall of plants and cancer?"

the common garden balsam and of Tropæolum to show at the top of the plant tumors covered with roots, of Ricinus and daisy to show tumor strands, of rose to show return of tumors after destruction, of okra to show encysted tumors becoming malignant, and of sunflower to show invasion of normal tissue by the stroma and other elements of the tumor. It deals chiefly with the result of efforts: (1) To produce these peculiar hyperplasias in the middle of internodes remote from axillary buds and where no shoots ordinarily develop;[3] (2) to determine under what conditions the tumors can be made to grow as ordinary sarcomata destitute of teratoid elements, or can be made to produce (a) roots, (b) leafy shoots, (c) floral abortions, or (d) a mixture of all of these teratoid elements; (3) to determine what particular tissues of the young internode (cortex, cambium, pith, etc.) may give rise to the teratoids, and what can produce only sarcomata; (4) to record the inception and progress of these tumors by means of good photographs, so as to bring before the reader clearly their astonishing proliferation and equally rapid decay; (5) to demonstrate by photomicrographs made from stained serial sections the embryonic and fragmentary nature of the teratoid elements occurring in the depths of these tumors; and (6) the existence of jumbled sarcomatous elements in their vicinity.

Incidentally some interesting records have been obtained on fasciation and various related abnormalities (flattening and fusion of organs or imperfect development of parts), on variability in rate of tumor growth, on secondary infections, and on failure of tumors once started to continue to grow. The last experiment on tobacco (September 7, 1916) also shows the germicidal effect of collodion used to cover the wounded surface immediately after the bacterial inoculation. The photographs speak for themselves and only a modicum of text is necessary.

## Experiments on Tobacco.

### A. In the Hothouse.

The plants used for these experiments exclusive of some broad-leaved, tall, ornamental, white-flowered tobaccoes (*Nicotiana sylvestris*), which also yielded teratoids but which will not be referred to again here, were hybrid (Connecticut-grown Havana seed) hothouse specimens of the Sumatran wrapper leaf type of *Nicotiana tabacum*. Some were grown in large pots; others had rooted through the bottom of smaller pots into a central bed. All of the inoculations here described, except those on the plant first figured (Fig. 1), which were in part at least into the leaf axils, were made on the main axis of the plant *in the middle of internodes, i. e.*, remote from buds. For the non-botanical reader it may be said that the stem or main axis consists of *nodes* and *internodes*. The leaves arise at the nodes, wherein there is a great interweaving and anastomosing of vessels, and in each leaf axil there is a dormant bud capable of growing into a shoot if conditions are favorable. These buds presumably furnish the teratoid elements of axillary tumors such as those already described (*l. c.*, Figs. 66 and 76) and those shown in Fig. 1 of this paper. In the internodes, on the contrary, the vessels run parallel to each other and to the longer axis of the stem in sheathing organs known as the wood cylinder (xylem) and as the inner bark cylinder (phloem). The bark cylinder is surrounded by a conjunctive tissue called *cortex*, and the inner (wood) cylinder is filled with a conjunctive tissue called *pith*. The two cylinders (of wood and of bark) are separated by a third thin cylinder of meristem known as *cambium*. This triple cylinder (phloem, cambium, and wood) is not a unit but a complex composed of many distinct groups of vessels known as vascular bundles. The vascular bundles in young stems radiate from the pith to the cortex like spokes from the hub of a wheel (see text figure), and each, therefore, includes its own portion of inner phloem (wanting in many species) protoxylem, xylem, cambium, phloem, and protophloem. Standing in the cortex, usually next to the phloem, are also in many plants bundles of strengthening fibers known as hard bast or bast fibers. These vascular bundles are separated radially by thin wedges of conjunctive tissue known as medullary rays, which in young stems begin in the pith and end in the cortex and which are added to tangentially from either side of the medial (cambium) line just as are the vascular bundles. In stems more than a year old new wedges of conjunctive tissue, secondary medullary rays, are intercalated from the cambium, and these, of course, do not reach to the pith. In very young stems the cortex is covered by a delicate living membrane (the epidermis); in older stems the epidermis is replaced by a many-layered, impervious, tough membrane (cork). As stems grow, numerous cork cambiums are formed cutting out peripheral portions of the stems and forming dead protective layers, often of considerable thickness (the outer bark). In some plants, *e. g.*, tobacco, which are then said to have bi-collateral bundles, there are two phloems, *i. e.*, in addition to the ordinary phloem outside the cambium there is an inner phloem lying between the pith and the wood (see text figure, p. 288).

The internodes selected for the experiments were severed about midway of the nodes at right angles to the longer axis of the stem by means of a sharp razor and crown-gall bacteria from young agar-streak cultures (many removes from the original colony) were pricked into the cut surface (not injected) by means of a delicate steel needle. The needle-pricks varied in depth from 1 to 3 mm. The organism used for all of the experiments detailed in this paper was the hop strain of *Bacterium tumefaciens* isolated in January, 1908, and cultivated in my laboratory on various media for eight years without inoculation (this particular line of the descent), then inoculated by the writer into sunflower (in 1915) with the production of tumors, from one of which it was re-isolated by means of an agar-poured plate and subcultured from a single colony called "Sunflower Colony 1." In all cases the inoculations were made from young agar-streak cultures two to six days old.

[3] See the *Journal of Cancer Research*, Vol. I, April, 1916, Plate XIX, Fig. 71, and context, where the first internodal tumor of this type is figured and described.

Done out-of-door on large, but slow-growing plants (Fig. 49), exposed to the hot sun, to dry air and to insect depredations, these experiments failed almost completely (in 21 out of 51 shoots, entirely), but in the clean, moist hothouse failures on soft, well-nourished plants were exceptional, *but were not exceptional on slow-growing firm ones.*

Fig. 2 illustrates the type of stem selected for inoculation (at *X*). Fig. 3 shows the appearance of one of the checks at the end of four weeks when a new top has developed from an axillary bud below at the left of the main axis, which meanwhile has trebled in diameter. No growth has taken place from the cut surface, and no growth ever does take place from such cut surfaces, as everyone knows who has ever had to do with the cultivation and pruning of tobacco.[1] When, however, such internodal cut surfaces are inoculated with pure cultures of *Bacterium tumefaciens* S. and T., they develop tumors of two sorts in varying numbers and with variable degrees of rapidity, the kind of tumor depending on the particular tissues stimulated. One type in my judgment is a plain sarcoma (the ordinary crown gall); the other is a sarcoma filled with proliferating teratoid elements especially with abortive leafy shoots, frequently to the number of several hundred.

[1] But the tendency is always there, if the stimulus is strong enough, not only in tobacco but probably in all other plants, and presumably also in all animals, since a great many plants and animals are known to give rise readily to adventive buds even under a slight stimulus, *e. g.*, willows and begonias; hydras and tubularias. Just what the stimulus is in many cases remains to be worked out. The shoots are especially apt to appear when the plant or animal has been wounded or otherwise thrown out of balance. Such shoots are normal, or pathological only to the extent of being more or less fused, crowded, distorted and starved. For these reasons some of them should undoubtedly be classed as typical (non-cancerous) teratoids. Several interesting cases in plants have come to the writer's attention lately and will be described elsewhere. These are on tomato, cauliflower, cabbage, pond lily, etc. In one very curious variety of begonia (*Begonia phillomaniaca*) buds in great numbers grow out of many parts of the leaf and stem on the slightest provocation (*vide* figures in Bailey's "Cyclopedia of American Horticulture," 1st ed., Vol. I, p. 146, and observations by Bateson in his "Problems of Genetics," pp. 50-51, where the species is considered to be a hybrid). In a begonia of this variety now growing in one of our houses, on an upper branch of the main axis (which was cut away), a single lower leaf has developed more than 600 of these diminutive shoots (embryo plants), but the remaining leaves on this shoot have developed only a few, the average number per leaf being 23. On this plant, their number is roughly proportional to the age of the organ bearing them, that is, proceeding from the lowermost leaf to the uppermost one the numbers of adventive shoots are 616, 56, 35, 0, 0. The same law holds good for the shoot axis bearing these leaves. It is 6 inches long and the adventive shoots from its surface are: Lower 2 inches, 56 (nearly all from lower half); middle 2 inches, 15; upper 2 inches, 2. But I have other plants the middle leaves of which bare most shoots. These are cuttings of last winter. Possibly loss of water due to the cutting may have caused this excessive germination of totipotent cells in particular leaves. All the leaves might lose water equally, or even the older ones most, and yet only the younger ones (topmost at time of cutting) be in condition to send out great numbers of shoots, *i. e.*, sufficiently meristematic to respond freely to the stimulus (see Mechanism of Tumor Growth, *l. c.*).

My first series of hothouse inoculations using the cut surface of tobacco internodes was made on May 31, 1916; my second on July 7; my third on July 29; my fourth on August 1; my fifth on September 7. The following is a brief account of these experiments, all internodal be it understood:

I. *Inoculations of May 31.*—All but one of the 12 inoculations of May 31 (from six-day agar) succeeded. Some gave rise to tumors with great rapidity (Figs. 4-7); others on plants of the same age and size and which appeared to be equally suitable for the inoculation gave rise to tumors very slowly (Figs. 8-10) and the tumors on several of the inoculated plants ceased to grow after some weeks and finally died (Fig. 12). All the inoculations were made into lower internodes long before the full development of the plant. Most gave teratoid tumors. The teratoid elements in these were abortive leafy shoots. No flower buds developed and no roots from any of these tumors, but frequently roots were stimulated to grow from the stem below the tumor both in these (Fig. 13) and in the subsequent inoculations. None of the leafy shoots that developed from these tumors were able to maintain the physiological functions of the plant. These were cared for by one or two strong shoots which developed normally from the lower leaf axils after the top was removed for the inoculation and considerably in advance of the appearance of the tumors. The tumors grew freely from all inoculated parts of the cut surface except the pith, but, so far as could be determined without making sections, the teratoid elements developed exclusively in tumors situated over the junction of wood and pith, *i. e.*, over the inner phloem region (Figs. 15-17); but not enough sections were cut to determine in every case their exact origin. In one plant (Fig. 18) two teratoid tumors were found to be outgrowths of the cambium. It is fairly certain that none of the many cortex tumors developed any teratoid elements unless it be the one shown at the left in Fig. 18. The leafy shoots grew out of the substance of the tumors, not out of the stem in their vicinity, and many of their leaves were defective in various ways (Fig. 6). All of these plants were in pots, but were vigorous and free from insect and fungous parasites.

II. *Inoculations of July 7.*—The inoculations of July 7 were from four-day agar cultures and were on taller, ranker plants which had rooted through the bottom of the rather small pots into a deep central bed. The cut internodes were softer than in Series I and some of them dried out without yielding tumors; but 15 of the 19 inoculations succeeded. In this case all the cut surfaces were covered lightly with collodion immediately after the inoculations (see Series V). The plants were from four to five feet high and nearly ready to develop blossom buds. Some of the internodes selected for inoculation were midway of these tall plants and others were near the top, but up to that time no flower buds had appeared. One of the upper inoculations yielded a teratoid tumor containing abortive flower buds as well as leafy shoots (Figs. 19, 20). The lower inoculations yielded many ordinary crown galls (sarcomas) and also teratoids in a number of instances, but the teratoid tumors contained only abortive leafy shoots, not flower buds. Here again the teratoid tumors seemed to arise from the region

of the protoxylem or that of the inner phloem, *i. e.*, the shoots were in tumors that developed over the pith periphery. The stems inoculated exclusively in the pith gave no tumors; those inoculated in the cortex yielded only sarcomas (Fig. 21). Concerning the cambium I was not so certain, because it is a narrow line on cross-section and even when working under a hand lens it is difficult in a long series of needle-pricks always to puncture that tissue only because sometimes a few minutes after it has been cut it cannot be seen clearly. The cambium tumors seemed, however, to be sarcomas, so far as one could judge origin by location in early stages of development. It is conceivable that the cambium might yield either sarcomas or teratoids, the difference depending on the particular cells inoculated, that is, on whether the infected needle entered the region of conjunctive-tissue cells or of multipotent or totipotent cells. Some of the teratoids developed shoots several inches long provided with leaves one to three inches in length (Figs. 22 and 25), but in all cases such shoots were tumefied (sarcomatous) at the base. Often on other parts of the same tumor there were close-set masses of pale-green buds (50 to 100 or more) which never developed further (top of Fig. 25). Not infrequently, both in this series and in the next, the teratoid elements over the entire tumor were of this second type and aborted very early (Fig. 27), never producing well-defined leafy shoots; nor did the tumors themselves in such cases resist decay beyond five or six weeks (Fig. 28, top row). Even in the tumors which grew rapidly and developed luxuriant leafy shoots there was always something wrong with the latter, *e. g.*, they grew rapidly for a short time only, were swollen at the base or throughout (*i. e.*, contained sarcomatous tissue), or were monstrous (distorted and fused with other shoots), feebly vascularized, pale green, and unable to develop beyond a soft, young stage. The best of these shoots died within three or four months, and decay of the body of the tumor soon followed. Often decay of central portions set in much earlier (Figs. 23 and 26 and tumor of the main axis on Fig. 29).

The flower buds which developed out of the tumor (Figs. 19, 20) contained stamens and pistils, but none of them came to maturity. All the buds dropped off one by one before the corolla opened.

In every plant, as in the first series, a new top was developed out of normal buds lower down, the leafy shoots in the tumor not being able to serve the needs of the plant. Some of these new tops were used for the third set of inoculations.

III. *Inoculations of July 29.*—The inoculations of July 29, being high on the plants, which were now in blossom, many teratoids bearing abortive flower buds were expected, but only leafy ones were obtained, that is, although there must have been "flower stuff" in the main axis at or near the level of the origin of these shoots it did not enter into them, the latter, at least where inoculated, containing exclusively "stem stuff" and "leaf stuff." The branches to be inoculated were cut at about midway of their length, in the middle of internodes as before. The 15 inoculations were by needle-pricks from two-day agar cultures. All succeeded. Nearly all gave teratoids, and most of these grew rapidly (Figs. 28, bottom, and 29, top), but two or three lagged far behind the others for no apparent reason, the plants being equally well developed. This contrast in amount of growth was well shown at the end of five weeks in two plants inoculated on the same date from the same culture and growing side by side, both tumors being teratoids, but one a thousand times as large as the other. It was also shown conspicuously in other plants (see Fig. 32, made toward the end of the seventh week, where only the larger tumor is a teratoid).

Here also in several instances, as was clearly visible in early stages of growth, the tumors bearing shoots were exclusively those which developed over the region of the pith periphery, but not enough sections were cut to be certain of their exact origin.

As in the second series, two types of teratoids grading into each other appeared. In the one type several relatively large, soft shoots developed along with several to many smaller ones (Fig. 28, V). In the other type there appeared no large shoots, but several hundred small shoots—distinct pale green centers of growth which were all of about the same size and which seldom passed beyond the stage of buds (Fig. 28, IV). On the surface of one tumor of this type 469 of these abortive shoots were counted and one lobe of this tumor is shown (enlarged) in Fig. 30 and a stained section of it containing numerous centers of embryonic growth in Fig. 31. As in Series II, both types of shoots frequently occurred on the same tumor. The rapid decay of these soft tumors is also illustrated in Fig. 28 (made September 13) where the upper row shows three dead tumors of Series II (68 days), and the lower row, two living ones of Series III (46 days).

IV. *Inoculations of August 1.*—The inoculations of August 1 were done at the same time, in the same manner, and from the same cultures (six-day agar) as the second series on Pelargonium, and were in the same hothouse. They were done on the tops of five tall, rather spindling (crowded) firm tobacco plants rooted through the bottom of the pots into a central bed and not yet in blossom. They were in two lots:

(1) Five main axis cross-cut internodes were needle-punctured on the cut surface in various tissues.

(2) Five cut midribs were needle-punctured in various tissues of the cut end which was within an inch or two of the junction of the leaf with the stem.

*Results.*—On September 15 (46th day) the results of the inoculations were as follows:

(1) *Stems.*—Two failed; three produced minute tumors. One of the internodes showed six very small tumors from as many needle-pricks, two of which were teratoids.

(2) *Midribs.*—All failed. The failures were due, I think, to early loss of turgor owing to evaporation from the cut surface.

In all respects these inoculations behaved like the out-of-door inoculations to be described later, although the plants were in the same hothouse, on the same central bed and treated in the same way as those which yielded the very successful inoculations of Series II and III. Series I was in another house.

V. *Inoculations of September 7.*—The inoculations of September 7, in the same house as Series I, were made on 28 young, clean, vigorous tobaccoes standing in 12-inch pots. They were from two to three feet high with many long and broad leaves. The stems at the base were an inch or more in diameter. They were cut off for inoculation at from one to two feet from the ground, and the upper three leaves also were removed except a small part, leaving five or six big, lower leaves (a foot or more in breadth and 1½ to 2 feet in length) to carry the plant. The cuts were in the middle of soft internodes varying in diameter from ½ to ¾ inch, and the shallow inoculations were made from 48-hour agar streaks, the depth of the numerous punctures varying from 1 to 2 mm. This experiment was planned with special reference to determining more definitely in which tissue the teratoid tumors originate. All of the inoculations were made under a hand lens, great care being taken to puncture only selected definite areas. The plants were in fine condition and I expected striking results. The 10 plants of the west row (right side of plate) were pricked all around the cortex and in the middle part of the pith. The nine plants of the middle row were pricked in the cambium region all around, but occasionally the needle (especially its tip) may have entered a little to one side or the other of this narrow ring of tissue. The nine plants of the east row were pricked in the outer part of the pith where it joins the vascular cylinder (inner phloem region). The needle used was one having the extreme tip bent at right angles to the shaft, so that only very shallow punctures could be made and great care was taken to puncture on a line parallel with the longer axis of the internode. The cut surface was shaded from the sun during the inoculations and immediately after the wet surface was wiped dry with a tobacco leaf and covered copiously with collodion, over which tobacco leaves were placed as a screen from the hot sun until the evening of the next day. The layer of collodion curled up and fell off after a few days, but it served its purpose, namely, to keep in the moisture of the cut surface until the plant could protect itself by the formation of a cork layer across the soft pith, and also much more than its purpose as subsequent results demonstrated, *i. e.*, it not only protected the cut surface from loss of water, but also destroyed the majority of the inoculated organisms and materially changed the conditions of the experiment. Strong shoots began to develop at once from axillary buds near the top of the cut stem and were several inches long at the end of 12 days (Fig. 33), while, much to my surprise, no tumors were yet visible. Also on September 29 (22d day), no tumors were visible. Even on October 20 (end of 43d day), only one tumor was visible—a small cambium teratoid. On November 12 to 14 conditions were as follows:

(1) *Cortex.*—One tumor on each of four plants. Two of them are bursting out below rather than through the cut surface as if only the more deeply lodged bacteria had been effective.

(2) *Cambium.*—One tumor on each of three plants. Two of them are bursting out below instead of through the cut surface as is usual in such inoculations.

(3) *Protoxylem Region.*—One tumor on each of three plants. All are teratoids.

*Result.*—Ten tumors only at the end of 68 days and all of these very small. An astonishing result considering the vigor of the plants inoculated, the virulence of the culture and the number of needle-pricks.

The final results obtained at the end of 4½ months were as follows:

(1) *Cortex and Pith Punctures.*—The cortex yielded five very slow-growing tumors, three of which were teratoids (Figs. 39 and 47). None of the pith punctures gave any tumors.

(2) *Cambium Punctures.*—The cambium yielded five very slow-growing tumors, all of which were teratoids (Figs. 44, 46*b* and *c*). The largest of these cambium teratoids is shown in Fig. 46*b*, and the smallest in Fig. 46*c*. The others were intermediate, but much smaller than the one shown in Fig. 46*b*, the best one being exhibited December 18, 1916, before The Johns Hopkins Medical Society and afterwards given to Dr. Winternitz. This was not photographed.

(3) *Protoxylem and Pith-Periphery Punctures.*—The protoxylem region yielded five very slow-growing tumors, all of which were teratoids (Figs. 34, 46*a*).

Considering the rapid-growing succulent nature of the stems inoculated (Fig. 33), these results were very different from those anticipated, since *out of approximately 375 needle-punctures only 15 (4 per cent) yielded tumors, and very slow-growing ones at that, while on 13 of the 28 plants no tumors whatever developed.* Moreover, the tumors started off so very slowly that for a number of weeks I thought the experiment was destined to be an entire failure. Finally, when the tumors did begin to show, they appeared first not on the cut surface as in all the previous experiments, but as swellings in the deeper parts of the tissues, *i. e.*, those remotest from the surface and farthest from the reach of the ether of the collodion; in other words the outer surface of all the needle-pricks and the depths of most of them were disinfected at the start by the excessive application of the collodion. As it finally turned out, however, the experiment is one of the most interesting in the series. Its results will be the more striking if we compare them with those of all the other inoculations detailed in this paper (excluding, of course, the slow-growing garden experiments on tobacco and the similar house experiment No. IV). We then have for comparison the following:

Tobacco experiments I, II and III—46 inoculations, 90 per cent successful.

Pelargonium experiments I, II and III—100 inoculations, 100 per cent successful.

Ricinus experiments I, II and III—75 inoculations, 100 per cent successful.

Okra experiments—25 inoculations, 100 per cent successful.

Balsam experiments—12 plants, about 50 groups of punctures, 100 per cent successful.

Tropaeolum experiments—15 inoculations, 100 per cent successful.

Brassica experiments—10 inoculations, 100 per cent successful.

*Total, 321 inoculations, successful, 98+ per cent.*

Serial sections from these tumors show that the teratoids may arise in tobacco either from the ordinary cambium or from a regenerative tissue at the junction of wood and pith, that is, from the region of the inner phloem, usually from its outer face (Figs. 35-38, 40-43, and 47, 48), where under the action of the parasite wood-vessels (tracheæ) are produced.

Concerning the three cortex inoculations which also gave rise to teratoids, the evidence from the sections is that two of these are developments from the cambium. It might be either that in these instances my needle wounded cambium as well as cortex, or, if not, that subsequently the overgrowth of the cortex tumor penetrated into and involved the cambium region and thus induced it to form leafy shoots. Sections from one of the tumors seem rather clearly to indicate the latter origin of the teratoid elements. Concerning the third teratoid, said to be cortical, the needle-puncture which produced it must have entered the region of the inner phloem (Figs. 47, 48), for it is that part which has proliferated.

### B. IN A GARDEN.

Observations of September 14 to 19, 1916, on internodal (mostly main axis) tobacco inoculations done in August on plants growing in good earth out-of-doors on the grounds of the United States Department of Agriculture (Fig. 49).

I. *Inoculations of August 1 from six-day agar.*

*Results.—a. On cut internodes.* One failed, eight stems gave tumors. All were very small (under 3 mm. in diameter) and not one was a teratoid.

*b. On ends of cut midribs near the stem.* Five failed; three were missing.

II. *Inoculations of August 5 from three-day agar, all into ends of cut internodes.*

*Results.*—The results may be briefed as follows: Twenty failed to produce tumors; two were missing or overlooked; 22 produced from one to a dozen small tumors (1 to 2 mm. or less in diameter).

These tumors were astonishingly small (Fig. 50) in comparison with those produced on the softer and better fed plants in the houses. Of these 22, only five produced teratoid elements (tiny green shoots) as follows:

*a.* No. 16. Plant inoculated high up for blossom teratoids.

*b.* No. 21. Inoculated in protoxylem region. One small tumor has developed. It is clearly a teratoid (shows tiny green leaves), and just as clearly it is now growing well within the vascular ring (Fig. 50*a*). Longitudinal sections of the stem bearing this tumor were made and these show the vessels of the tumor to have their origin from the inner phloem region of the stem (Fig. 51).

*c.* No. 27. Marked "Inoculated mostly in cambium." It bears an interrupted ring of 12 tiny tumors (probably from as many needle pricks), two of which are teratoids, *i. e.*, bear minute green shoots. These two tumors are the innermost ones (top and bottom of third stem in first row of Fig. 50).

*d.* No. 39. Marked "Inoculated in the cambium, approximately." Bears several small tumors among which are three tiny teratoids which seem to arise from the cambium.

*e.* No. 43. Inoculated in the protoxylem region. One tiny tumor which bears a green shoot is well within the vascular ring. Longitudinal sections show this tumor to have originated from the inner phloem region. A smaller teratoid tumor on the opposite side of the stem arises from the cambium; a third very small tumor which is not a teratoid grows from the cortex (conjunctive tissue). Not photographed.

The most striking result of this experiment, as of the preceding, is the insignificance of the tumors considering the time that has elapsed (six weeks) and the size of the plants which are leafy and six to seven feet high (Fig. 49).

I cannot help thinking, not only as a result of these experiments, but also of many others, that over-nutrition with its resultant rapid growth and soft tissues greatly favors the development of these tumors, whereas moderate feeding, which induces a slow firm growth, discourages them. They do not occur in the absence of the bacteria.

### Experiments on Pelargonium.

When it had been settled that atypical teratoid tumors could be produced from the midribs and the internodes of tobacco, I turned my attention to the leaves and internodes of the common cultivated geranium (*Pelargonium zonale*) selecting a double, red-flowered variety which had previously given teratoids abundantly when inoculated in the leaf axils (Fig. 52).[3] All of the plants used for these internodal inoculations were hothouse-grown specimens and were in good condition, free from insects and fungous parasites. My first inoculations were made on July 7, my second on August 1, and my third on September 29.

I. *Inoculations of July 7.*—The first plants used were rather tall, straggling plants inclined to produce foliage rather than flowers, since they stood on a central bed and their roots had grown through the bottom of the pots into the deep earth. The inoculations were made on the cut surface of internodes at the same time, in the same manner, and from the same cultures (four-day agar) as in the second series of tobaccoes. The stems were cut off well toward the top of the plant (about two feet from the ground) and were inoculated in the pith as well as in the other tissues, since I wished to know whether the young pith would proliferate. All of these 18 internodes gave tumors freely over the whole or nearly the whole inoculated surface including the pith, and most of them were teratoids, leafy shoots, however, being more abundant in them than floral abortions, although in places sparingly four of them developed small, irregular outgrowths showing the red color of the flowers. Photographs of several of these teratoids are shown in Figs. 53, 54, 56 and 57. None of the teratoid elements was developed out of the pith, and none, I believe, came from the cortex or from the protoxylem region, there being no inner phloem in the Pelargonium. Of the whole number of inoculations into shoots none failed, but some of the tumors grew

[3] See also *The Journal of Agricultural Research*, April 24, 1916, Plate XVIII; and *The Journal of Cancer Research*, April, 1916, Plates XIV and XV.

slowly, *e. g.*, Fig. 55, which showed no teratoid elements. In the two stem ends which were sectioned in early stages of the tumor-growth, the teratoid elements clearly were developed from the cambium, while the non-teratoid tumors on the same stem were from the cortex, or cortex and cambium (Figs. 58 and 59). In nearly all of these teratoid tumors there was more or less fasciation of the leaves and shoots. See, for instance, the tallest leaf in Fig. 54*a*, the blade of which is fused into a cup. Generally about two months after their appearance, but sometimes earlier, the teratoid elements died (Fig. 54*b*), and the tumors themselves soon after softened centrally and rotted. No definite flower buds appeared such as those shown in Fig. 19, but only numerous tiny, twisted outgrowths containing the red flower pigment. In other respects, *i. e.*, except for the occurrence of pith proliferation and the absence of normally formed flower-buds, this experiment duplicates in all respects the corresponding one on the tobacco and shows that teratoid tumors full of abortive leafy shoots and exhibiting variable rates of growth can also be produced from the cut internodes of the Pelargonium, *i. e., from places where normally no shoots ever appear.* Roots, however, grow out of such cut surfaces if they are buried in the earth, and this is the gardener's method of multiplying geraniums (Fig. 63).

II. *Inoculations of August 1.*—The second experiment was in all respects a duplication of the first, and was done on the same group of plants. The results were the same, to wit, the pith as well as other portions of each one of the 16 inoculated internodes proliferated, the whole top being covered and overgrown with tumor tissue (Figs. 60 and 61). All not only contracted the disease, but all produced abortive leafy shoots and five also produced red pigment (flower color) in places. One of these red patches (back of No. II, Fig. 60) was cut out and fixed on September 6 and afterwards sectioned and photographed (Fig. 80). All were cut early (September 13) and photographed on one plate (Figs. 60, 61), at which time (44th day) all but one (No. VIII) were living.

The teratoid elements grew slowly as compared with those on the tobaccoes of Series I, II and III, and often a considerable period elapsed before they became evident, *i. e.*, there was not much evidence of green leafy shoots in these tumors until about the beginning of September (30 days). None of these shoots developed from the pith, most were well out toward the periphery of the tumor (cambium region). Frequently there was more or less fusion of neighboring parts normally separate (fasciation). See, for example, Fig. 61, XVI, where the photograph shows a fused twisted petiole, bearing two leaf-blades. The same kind of fusion occurs at *X* on Fig. 60, VI, but is not distinct in the photograph. Tumors II and III on Fig. 60 and XI on Fig. 61 also bear fused masses of leaf buds comparable to those which appeared on some of the tobacco teratoids, *e. g.*, Figs. 1 and 25.

III. *Inoculations of September 29, 1916.*—The third experiment was made exclusively on young leaves (blade and petiole) to see if teratoids could also be obtained from these parts, as had been done on tobacco.[6] About a dozen leaves were inoculated and six of them produced small tumors bearing leafy shoots. One was on a petiole (Fig. 62) and the others were on the leaf-blade. The best developed were picked off by some one before photographs had been made.

In Fig. 63 are shown crown-gall teratoids, or what I believe to be such, occurring naturally on two Pelargonium cuttings. These originated on the propagating bench of a florist's establishment near Baltimore and were received in March, 1917, through the courtesy of Mr. Philip Garman, of the Maryland Agricultural Experiment Station. Poured plates were made from the base of Plant I after surface treatment with mercuric chloride, and white colonies were obtained on agar-poured plates which appeared to be crown-gall colonies, that is, they looked typical. Five of these were selected, subcultured, and inoculated into Ricinus, Pelargonium and tobacco, but all failed. Had we been less certain at the start we would have subcultured from a much larger number of colonies and then very likely some of them would have proved infective. The writer has used Pelargonium for crown-gall inoculations many years, but this is the first time any natural tumors have been seen on the plant. In both instances the tumors started from the base of stunted cuttings, *i. e.*, in the knife wound, and probably from contact with infected soil. The tumor on No. II has developed both roots and shoots.[7] Further details on Pelargonium tumors will be given under "Inner Structure of the Teratoids."

## Experiments on Ricinus.

The experiments on *Ricinus communis*, the common castor oil plant, are a continuation of those mentioned in *The Journal of Agricultural Research* (April 24, 1916), as just begun. Inoculations I and II were all done into the leaf axils and most of them developed teratoids, but always with fewer shoots than those on the tobacco or Pelargonium. The tumors grew with great rapidity to a diameter of several inches, decayed early, and were very destructive to the plants. The inoculations mentioned under Series III were done on young leaf-blades. Owing to the ease and certainty of inoculations and the short time required to grow plants from the seed, *Ricinus communis* is a good plant both for demonstrations and for controls when working on untried species, provided it can be grown under good hothouse conditions.

I. *Inoculations of March 17, 1916.*—These were by needle-pricks introducing the hop strain of *Bacterium tumefaciens* (from four-day agar streaks) which had been passed through

[6] For midrib and other rib teratoids on tobacco leaves, see especially *The Journal of Agricultural Research*, April 24, 1916, Plate XXIII and text; but also *The Journal of Cancer Research*, April, 1916, Plates XIX, XXI, and XXII.

[7] Two months later, another galled cutting was obtained from the same source. Plates were poured and a small number of colonies appeared. Twelve of these colonies, which looked entirely typical for the crown-gall organism, were sub-cultured and each inoculated into several plants of Ricinus and Pelargonium, but without positive results.

sunflower in 1915. All were successful, but I shall not here take the space to describe them in detail. The nature of the results obtained are shown substantially in the three photographs (Figs. 64 to 66) including occasional formation of roots on stems below tumors (Fig. 65). On the plant shown in Fig. 66 there was, on the tumor at the left, a conspicuous crowding, stunting, and fasciation of leafy shoots. These tumors, like the okra tumors, to be described later, were first covered with cortex, but later the sarcomatous elements ruptured to the surface conspicuously, as at *x, y, z* in Fig. 66. In Fig. 64, at the right, a portion of the teratoid growth of the tumor has become sarcomatous (petiole at *x*, and below *x* in its interior).

II. *Inoculations of March 21, 1916.*—These plants were of the same age as those in Series I and were inoculated in the same way with the same strain of the crown-gall organism, a four-day agar-streak culture being used. All the inoculations were successful, most yielded teratoids, and the disastrous effect on one of the plants is shown in Fig. 67. These two plants stood in the same pot, were of the same age and size when the left-hand one was inoculated, and the subsequent difference in behavior is to be ascribed solely to the effects of the inoculation, *i. e.*, to the development of the two tumors. Many of these tumors also were smooth at first, *i. e.*, covered with a normal membrane (epidermis) and with normally arranged subepidermal tissues, but afterwards the rough sarcomatous element ruptured through just as shown in Figs. 64 and 66.

III. *Inoculations of March 21, 1916.*—This experiment was an effort to produce teratoids from the leaf-blade of Ricinus. The leaves were punctured with the infected needle when young, especially in the middle of the blade where it joins the petiole. Many tumors developed but none of them bore shoots. This is, however, only a single series and the inoculations should be repeated many times, especially on very young leaves before we may conclude definitely that teratoids cannot be produced from the leaves of Ricinus, especially as cells capable of such growth appear from the preceding experiments to be much less abundantly distributed in Ricinus than in tobacco or Pelargonium.

It was one of the leaves in this series (collected May 19) that yielded the tumor-strand referred to in my paper in *Science* (*Science*, n. s., Vol. 43, p. 886). A cross-section of this tumor-strand 9 cm. below the primary tumor is shown in Figs. 68, 69. The strand is nearly surrounded by pith, but, as in daisy, it is included in the vascular bundle, *i. e.*, it occupies the extreme inner face of the bundle, the only elements separating it from the pith being the somewhat thickened (protective) sheath (bundle sheath?) and an occasional spiral vessel torn away from its fellows by the growth of the elements of the tumor which appear to have been derived from parenchymatous conjunctive elements (medullary ray cells) lying between the spiral vessels from which, however, that is, from normal ray cells, they stain quite differently. Numerous small petiole tumors developed from this strand, but none exhibited leafy shoots or had ruptured to the surface at the time the leaf was collected and fixed. In places there were slight penetrations of these tumors into the petiole cavity. That part of the woody cylinder of the leaf stalk adjacent to the tumor-strand, *i. e.*, on that side of the petiole was also much thickened (sextupled in diameter).

### Experiments on Okra.

The inoculations on common okra (*Hibiscus esculentus*) were made June 15, 1916, into the leaf axils of actively growing plants, and produced slow-growing tumors, some of which, at least, were teratoids (Fig. 70), although that element of the tumor was very feebly developed, much less so even than in Ricinus. They were produced with the hop strain of the crown-gall organism and are mentioned here chiefly as confirmatory of the last statement under Ricinus (Experiment II). Both the tumors here shown are encapsuled, *i. e.*, covered with cortex and cork, the right one entirely, the left, except in four places where the rough irregular naked sarcomatous growth has ruptured through and is fungating on the surface. Necrosis has begun at *X*. Fig. 71 shows the back side of the left (ruptured) tumor of Fig. 70. The action of the micro-organisms has been expended, as sections show (Figs. 72, 73), chiefly on the wood and the pith, and growth has been very slow, but the tumor is due to the same cause as the other crown galls here shown, only in these okra tumors the plant has had the advantage and has protected itself as much as possible. No secondary tumors were observed and growth was so far along when these tumors were collected, the plants being in fruit, that it is doubtful whether the sarcomatous element of tumors like the right-hand one in Fig. 70, of which there were many, would ever have ruptured to the surface.

### Experiments on Balsams.

Sometimes in Paris daisy,[8] tobacco (Fig. 13), Brassica, Coleus, and Ricinus (Fig. 65), feeble roots have been observed to develop from stems in the vicinity of crown galls, and crown galls themselves bearing roots are seen frequently on the apple (as hairy root) and have been produced by us on a number of plants with an organism isolated from the apple hairy root, but the tobacco and Pelargonium teratoids treated of in this paper, all of which were produced with the hop strain of the crown-gall organism, *i. e.*, with an organism isolated from a hop tumor which did not bear roots, developed only leafy and floral abortions.

However, the common garden balsam (*Impatiens Balsàmina*) when inoculated in the upper leaf axils with the same hop strain produced roots in abundance directly from the tumors (Fig. 74, made at blossoming time; and Fig. 75, some weeks later), so that here is unexpected confirmation of the view which I expressed in 1911,[9] that "hairy root" is not due to a distinct micro-organism, but is only a special kind of crown gall, the appearance in the tumor of roots being de-

[8] Bacteria in Relation to Plant Diseases. Carnegie Institution of Washington, Vol. II, Fig. 26.

[9] Bulletin 213, Bureau of Plant Industry, U. S. Dept. of Agriculture, page 157.

pendent on the infection either of the root anlage or of areas containing "root stuff." Some of these balsam teratoids also bore leafy shoots (Fig. 74, at *x;* Fig. 75, at *x* and *z*), and all apparently contained "flower stuff," judging from the color of the tumors which were red (roots included) on a pale green stem in all the varieties yielding pink, red or purple flowers, but not in those yielding white flowers. In this way it was possible to tell in advance of the development of blossoms whether the plant would produce white or colored flowers. Judging from these inoculations, balsams in all of the above-ground parts must be very abundantly provided with "root stuff" or with pluripotent cells capable of growing into roots, much more so than tobaccoes or Pelargoniums. Also under the stimulus of the micro-organism of crown-gall, as in Pelargonium, the floral color is anomalously distributed in the tumor, *i. e.*, it is generally diffused in the stem and root cortex. Common tobacco also has pink or reddish flowers, but I have seen no such abnormal distribution of floral color in its teratoids.

### Experiments on Tropæolum.

On August 5, 1916, a set of inoculations was also made into the common garden nasturtium (*Tropæolum majus*) with the hop strain of *Bacterium tumefaciens,* the inoculations being made into leaf axils (foreground of Fig. 49). These plants produced numerous tumors where they were inoculated and some, at least, of these tumors developed both roots and shoots (Figs. 76 and 106).

### Experiments on Brassica.

These experiments were on cabbage plants and cauliflower plants grown in the hothouse. Into these plants, generally believed to be derived from the same species (*Brassica oleracea*), inoculations into the leaf axils were made on September 29, 1916, the hop strain of the crown-gall organism being used; and tumors containing both shoots and roots were obtained. The shoots from these tumors were twisted, abortive, and swollen at the base, or sometimes sarcomatous throughout, and were generally whitish or with only a little leaf green. The appearance of an inoculated stem of cabbage bearing two tumors is shown in Fig. 77, and both the front and back view of an inoculated stem of cauliflower is shown in Figs. 78, 79.

### Inner Structure of the Teratoids.

The Pelargonium embryomas were the first discovered and up to this time they are the only ones that have been sectioned freely in series. The illustrations and comments, therefore, under this head will be confined mostly to the geranium, the equally interesting inner structure of the tobacco, balsam and other teratoids being left for future consideration.

With the exception of one internodal tumor (Figs. 80-87) the tumors used for these sections were axillary teratoids (like that shown in Fig. 52) and were produced by pure-culture inoculations, made in October, 1915, with the hop strain of *Bacterium tumefaciens.*

The material for the sections was collected February 28, 1916, fixed in Carnoy's solution (¾ ethyl alcohol, ¼ glacial acetic acid), embedded in paraffin, sectioned in series on the microtome, and suitably stained and counter-stained, generally a long time with methyl green for the woody parts and then a short time with acid fuchsin (for the parenchymatous parts).

In some cases in the depths of the Pelargonium tumors I have seen what I take to be floral anomalies, in other cases I have not identified the fragments, and in still others they are clearly vegetative (leafy) parts. These parts are frequently lined with membranes and lie unconformably in other tissues, often sidewise or upside down, as may be seen from the photomicrographs. These embryonic fragments all lie in the abnormally thickened cortical part of the tumor, never in the pith or xylem region, so far as observed. They have been seen in all stages of development. Whether on the surface or buried, these teratoid fragments are usually small, and they are not malignant except in the sense that they are under an abnormal and evil stimulus due to the rapidly proliferating, unripe sarcoma cells. Wherever situated, they are in all respects like other (normal) embryonic organs, except that they are fragmentary, as if cut off and displaced by the growth of the tumor, are very feebly vascularized, imperfectly nourished, and subject to early decay. Their embryonic nature is also shown by the small size of their cells, as compared with mature cells of the same sort of tissue, by their rich plasma, by their deep reaction to stains, and by what they develop into, all of which is evident on the photomicrographs. In any stage they are easily distinguished from the sarcoma by their unlike behavior toward stains and by their regularly co-ordinated cell structure. In the orderly arrangement of their cells the elements of the embryonic tissue in these tumors may be likened to the elements of a brick wall, whereas the elements of the sarcoma are almost as disorderly as those of a fallen wall (Figs. 38, 105). In some cases where two or three of these epidermal fragments come together with a small cavity between them, as in Figs. 90, 96, 97, they are somewhat suggestive of cysts.

Fig. 80 shows a vertical section through a Pelargonium teratoid produced on the cut surface of an internode by needle-pricks introducing *Bacterium tumefaciens.* The outer surface of the stem is at the left and the arrows indicate approximately the level of the cut surface, below which for a short distance there is a disturbance in both the cortex and the pith, but the principal development of the tumor has been from the region of the vascular bundle and both phloem and xylem are involved in it, the wood in particular being much thickened. The protoxylem region is not disturbed, nor is the deeper pith or cortex. At the top are teratoid elements. Many sections were cut through this tumor, which in this region showed floral color, and details of them at various levels are here shown, *e. g.,* Fig. 81 shows thickened and distorted cambium in the deeper part of the tumor, the outermost tracheæ (xylem vessels) being at the right and the sieve tubes (phloem) at the left.

Fig. 82 is from the same section but nearer the surface, *i. e.*, in the cortex of the tumor. It shows one of those nests of twisted cells and vessels that in crown gall so commonly take the place of the normally oriented tissues—a whorl of tissues with changed cortex cells at the center, curved tracheæ midway, and bent sieve tubes on the periphery. In crown galls on various plants, *e. g.*, the peach, these pearl-like nests of woody whorls can be shelled out of the soft surrounding parts of the tumor as smooth, irregular, hard nodules, round or more often tapering to a sharp point (first described by Toumey). Fig. 83 shows two smaller immature whorls of the same sort located one field of the microscope above Fig. 82.

Fig. 84 shows a lump of deep-staining sarcomatous tissue (with distorted tracheæ) lying in more or less disturbed cortex and connected by a coarser rather vague pedicle of cells (lower side and better developed in another section) to the main body of the sarcoma.

Fig. 85, from the same vicinity, shows in its center (white area) the beginning of tumor disintegration. The central cells in this area were shriveled and the surrounding ones dead at the time the material was fixed. This area has stained a feeble greenish white, but has no power to fix the red fuchsin stain. Fig. 86 shows sarcoma cells enclosing an island of unchanged (?) coarse-celled tissue (cortex ?), near the surface (top) of the tumor. The cells are much larger than those of ordinary cortex. Fig. 87 gives another portion of the sarcoma showing the small size and loss of orientation in its cells. This lies in the middle of the tumor and consists chiefly of rapidly dividing parenchyma cells among which are a few distorted vessels (tracheæ). The ratio of nuclear matter to cell cavity in normal cortex cells of tobacco or geranium is about as 1:500, but in rapidly dividing sarcoma cells, that is, in cells which cannot mature before another division occurs, the ratio is greatly reduced, being only about 1:20 or 1:30. In other words, the volume of nuclear material is greatly increased in the sarcoma.

Fig. 88 is from a solid axillary embryoma. It is a planar enlargement of a considerable fragment of the tumor, viz., of a whole microtome section, the surface of the tumor being at the left. The curved part from the center (*XY*) to the left represents the overgrown cortex in which, at or near the lettered places and elsewhere, are located the embryonic fragments, photomicrographs of some of which are shown in Figs. 90 to 99, Fig. 89 showing a small abortive surface organ. Many of these, as already stated, were red, *i. e.*, showed floral color. All the deeply staining parts of this section, whether lettered or not, contain embryonic tissues. Special attention is called to the fragmentary nature of these organs lying unconformably on other tissues (Figs. 90 to 92, 95), to the development of trichomes (hairs) on the epidermis of these buried fragments (Figs. 92, 94), to the orderly arrangement and characteristic staining of the component cells of these embryonic masses, and to the proximity of differently staining pseudo-embryonic and disorderly (sarcomatous) elements (Figs. 90, 92, 93).

Figs. 101 to 104, which are from another tumor, show a small tooth-shaped bud, normally arranged as to its cells, but lying bottom up in overgrown cortex and surrounded by sarcomatous elements. Especially in Fig. 105 (from the same series) the sharp border-line between sarcoma and overgrown, but normally arranged cortex, can be seen most beautifully. Figs. 101-104 and Fig. 105 are details of Figs. 65 and 64 in my paper in *The Journal of Cancer Research*, April, 1916, there insufficiently magnified, but which may be consulted for orientation. Fig. 105 is taken from the right side of Fig. 64 along the border-line of *B-B*. In Fig. 100, for comparison with 101, is shown the normal cortex of the Pelargonium stem covered with cork.

In Fig. 101 is shown the surface layer (enlarged cortex) of an embryoma covered by a membrane (epidermis) and bearing (at this level) five trichomes. It is a part of the tumor, *i. e.*, of the overgrowth, and its cells are smaller than the normal cortex cells, but they are arranged in an orderly way and are non-malignant except perhaps the deeper and dark-stained ones. One field of the microscope below this section (deeper in the tumor) is presented in Fig. 102, in the middle of which is the tooth-shaped piece of tissue (bud) covered with a membrane and having in its center embryo vessels, but with its apex pointed directly away from the surface of the plant, *i. e.*, toward the center of the tumor, the impulse to this abnormal development being lodged in the surrounding bacterially occupied sarcoma cells shown in the following plates (Figs. 103 to 105).

### Recapitulation of Crown-Gall Investigations (As Related to Cancer).

(1) Crown gall is a common tumor due to a white rod-shaped polar-flagellate schizomycete. It occurs on many kinds of plants, wild and cultivated, but chiefly on the latter.

(2) In certain striking ways, as I have previously pointed out, this tumor resembles malignant animal tumors.

(3) For earlier literature on its structure and etiology, and on the morphology and biology of the organism causing it, consult Bulletins 213 and 255 of the Bureau of Plant Industry, U. S. Department of Agriculture (price 40 and 50 cents), to be had from the Superintendent of Documents, Government Printing Office, Washington, D. C. In the *Journal of Cancer Research* (April, 1916) I have specially summarized my conception of its relation to human cancer, and in *Science* (n. s. June 23, 1916), I have endeavored to answer various objections to my views.

(4) The commonest form of the tumor is a sarcoma, that is, a hyperplasia developed out of conjunctive tissues. In 1906, for the first time, this tumor was produced with a definite micro-organism by the writer and his associates, since which time we have produced hundreds of crown galls with pure cultures of our *Bacterium tumefaciens*. Cancers occur, therefore, if my interpretation is correct, not only in animals, but also in plants.

(5) The parasite is intracellular and invisible, or at least very hard to demonstrate in tissues,[10] but can be isolated by the methods of the bacteriologist. It can also be isolated on slides, and stained, by diffusion in sterile water from the cut surface of fresh young tumors, and is then seen to be about the same size and shape as when grown in cultures. It is not very abundant in the tumor. It is not demonstrable in the vessels or between the cells.

(6) It does not kill the tissues, but stimulates them into abnormal growth by means of its diffusible products—acid and alkaline. (See "Mechanism of Tumor Growth in Crown Gall," *Journal of Agricultural Research*, January 29, 1917.) Death of tissues comes about in other ways, *i. e.*, by loss of water or by the destructive action of other organisms, the naked tumor offering unusual facilities for their entrance.

(7) The ordinary crown gall, as elsewhere described, gives rise to parenchymatic tumor strands on which secondary tumors develop with the structure of the mother tumor. Many of these secondary tumors have been seen especially on the Paris daisy, but so far as observed not one of them has been an embryoma.

(8) Many of the experimentally produced tumors here described are embryomas, that is, sarcomas containing rapidly developing abortive parts of the young plant—roots, stems, leaves and flower-buds or cells containing floral pigment.

(9) These tumors are caused by the same parasite as the ordinary crown galls, the difference between the two types of tumor being due to unlike reactions of the various tissues. If conjunctive tissue only is stimulated, a simple sarcoma results, but if, on the contrary, the infected conjunctive tissue cells are close to totipotent or pluripotent cells, then they also begin to grow and a complex tumor results—an embryoma.

(10) When the embryomas give rise to secondary tumors, the latter are either embryomas, like the mother tumor, or simple sarcomas. In this particular, also, they follow the law of animal embryomas (Askanazy).

(11) The organs or tissue fragments in the embryomas are feebly vascularized and abort at various stages of development, usually early.

(12) The organs and fragments of organs in these tumors are often monstrous, *i. e.*, simplified or reduced or duplicated, or fused, or abnormally oriented, or asymmetrical; and frequently their tissues also are subsequently invaded by the sarcoma.

(13) Monsters occur frequently in nature and have been produced repeatedly in various ways, *e. g.*, by grafting or by fragmentation of eggs, but are never sarcomatous or only accidentally so. This is the first time, so far as I know, that embryomas have been produced experimentally, and certainly the first time they have been produced by means of inoculations introducing a micro-organism.

[10] I now think the intracellular rods which we stained by means of gold chloride, and which I interpreted as the parasite, are mitochondria.

(14) Whether epitheliomas and carcinomas can also be produced in plants by bacterial inoculation remains to be determined, but I believe they can be. My reason for thinking so is the fact that I have obtained the first stages of cell division in epidermal cells by bacterial inoculation and see no reason to doubt that under favorable conditions the epidermis would continue to divide and would follow its own law of growth in tumor development, *i. e.*, downward into the subepidermal tissues. This must be left for further experiment.

(15) Frequently normal tissues are torn and crushed by the enlarging tumor (mass invasion), but there is also an individual invasion on the part of tumor cells and vessels (Fig. 115). I do not know, however, whether surrounding tissues are also absorbed.

## Some Definitions.

By request, and rather against my own inclination, I give some definitions which may perhaps tend to a better understanding of my text.

*Bark.*—Outer part of a stem from cambium cylinder to the surface. In young stems it consists of (a) phloem, (b) phloem rays, (c) cortex, (d) epidermis. Often the cortex also contains bast fibers and collenchyma, which are strengthening tissues. In older stems the outer part contains cork and various dead tissues of a protective nature.

*Bast.*—The inner part of the bark. Often distinguished as *hard* bast (= bundles of tough fibers) and *soft* bast (= the phloem).

*Bud.*—A group of dormant cells having the potentiality of a shoot; the anlage of a shoot. Buds are commonly found in the leaf axils.

*Adventitious Buds.*—Buds occurring in abnormal places, usually in the absence of definite anlage, and under some special stimulus.

*Cambium.*—Any generative layer, as cork-cambium. Used without qualification, the generative layer encircling the wood cylinder. Wood is produced from its inner face and bark from its outer face.

*Cells.*—Nodal points in an organism. The morphological units of the body. Growth takes place by division of cells, but every complex multicellular organism must be regarded as one thing no matter how many cells compose it, since all its parts are physiologically coordinated. Morphologically also the individual cells are not separate entities, but are connected in many instances by delicate bands of protoplasm. As we pass downward in the scale of development the coordination is less imperative.

*Unipotent.*—Said of cells capable of producing only one sort of tissue, *e. g.*, connective tissue.

*Multipotent* or *Pluripotent.*—Said of cells capable of producing several tissues or a considerable part of the body.

*Totipotent.*—Said of cells capable of producing the whole organism.

*Collenchyma.*—A protecting and strenghtening tissue found in the outer part of the cortex in many plants. It consists of groups of lengthened cells with thickened angles.

*Conjunctive Tissues.*—The connective tissues of the plant. See diagram.

*Cork.*—A firm, impervious, many-layered, protective tissue which takes the place of the epidermis on older stems.

*Cortex.*—The outer part of the living bark. The cylinder of parenchymatic cells lying between the phloem and the epidermis or its equivalent, *i. e.*, on the older stems the cork cylinder. Special cells of the cortex exhibit various secretory functions.

*Embryonic.*—Of or pertaining to the embryo plant or animal.

*Pseudo-Embryonic Tissue.*—Cancerous tissue in the broad meaning of that term. This tissue has taken on increased vegetative

activity, emancipated from physiological control, and at the same time has lost the ability to ripen and differentiate its cells. This is the most fundamental distinction between cancerous tissue and normal embryonic tissue.

*Epidermis.*—A living layer, one cell thick, covering young stems, leaves, etc. Its outer surface is protected by cutin, wax, etc. The plant equivalent of epithelium.

*Fasciation.*—A peculiar phenomenon not uncommon in plants, whereby a growing point flattens out as it grows or breaks up into several to many flattened fused shoots—a monstrous growth.

*Germ Cells.*—Those ovarian cells which, when fertilized or developed parthenogenetically, reproduce the entire organism. Possibly, also, cells or groups of cells widely distributed in the body (either normally or abnormally) and usually dormant but potentially able to reproduce the whole or a considerable part of the organism. Such cells are supposed by many persons to be the only ones capable of producing embryomas.

*Giant Cells.*—Cells containing several to many nuclei. Those not due to cancer are distinguished as "foreign body" giant cells, but this is probably a distinction without a difference. They are found in tuberculous nodules, in nematode galls, and in tissues reacting to the injection of various extraneous substances.

*Somatic Cells.*—The mass of cells making up the body of a plant or animal. All cells but germ cells. Terms used by Weissmann.

*Hyperplasia.*—A tumor due to increase in number of cells. All cancers are hyperplasias, but all hyperplasias are not cancers.

*Hypertrophy.*—An enlargement of individual cells.

*Medullary Rays.*—Conjunctive tissue separating the vascular bundles and, in stems of the first season, extending from cortex to pith. The silver grain of wood. It is developed from the cambium line. See radiating black lines in text figure.

*Phloem Ray.*—The outer, bark-part, of the medullary ray.

*Xylem Ray.*—The inner, wood-part, of the medullary ray.

*Meristem.*—Any cambium or generative layer.

*Mitochondria.*—Normal organs of the cell which recently have attracted much attention. They are self-motile. Often they are rod-shaped and divide like bacteria, which, frequently, they also resemble in size and in movement. Their function is unknown or still in dispute. (See a very good paper by Lewis and Lewis, *in* Amer. Journ. of Anatomy, 1915, XVII, No. 3, pp. 339-401.

*Node* and *Internode.*—The joints of a stem. See text.

*Pith.*—The inner part of the conjunctive tissue. It forms the center of stems, and in old stems it is a dead tissue.

*Phloem.*—Technical name for the inner part of the bark cylinder. It consists chiefly of sieve tubes and companion cells. It functions as a carrier (downward) of elaborated nitrogenous slime compounds. See *R* of text figure.

*Phloem Ray.*—(See medullary ray.)

*Plasma.—Protoplasm.*—The living contents of the cell outside the nucleus. It consists of a clear, non-motile, fatty surface membrane (hyaloplasm) which controls osmosis, and of an interior, granular motile polioplasm. In terms of colloidal chemistry the hyaloplasm is a *gel* and the polioplasm is a *sol.*

*Protophloem.*—Primary soft bast. The earliest phloem.

*Protoxylem.*—The primary wood consisting of ring vessels or spiral vessels. The earliest xylem. See *S* of text figure.

*Secondary Infection.*—As here used the invasion of a cancer by some parasite or saprophyte.

*Starch.*—A carbohydrate storage product corresponding to glycogen in the animal. The plant converts starch into sugar to meet needs of growth, and excess of sugar is generally stored as starch. There is often much starch in crown galls just as there is much glycogen in cancers.

*Stroma.*—The connective tissue and vessels of a tumor. In crown gall it consists of conjunctive tissue and two types of vessels—tracheæ from the xylem, and sieve tubes from the phloem.

*Stuff.*—Technical term introduced by Sachs to designate hypothetical reserve substances specially adapted for development of roots, shoots, etc.

*Trichomes.*—The hairs of plants. These are outgrowths from cells of the epidermis. They are either simple or compound. Many are glandular at base or apex.

*Turgor.*—Opposite of flaccidity; the state of being full of water. Without turgor no cell can divide.

*Wood.*—The lignified chief strengthening and supporting part of the stem, which acts also as a conductive and aerating tissue. It is composed mostly of thickened fibers and vessels permeated with a hard substance called lignin, but it also contains more or less parenchyma. Its earliest formed vessels next to the pith periphery are ring or spiral vessels. These very soon give place in the plants here considered to tracheæ (pitted or scalariform vessels), which are the only sort in the secondary wood. Tracheæ do not occur normally in cortex or pith but are produced there under the stimulus of the crown gall Schizomycete.

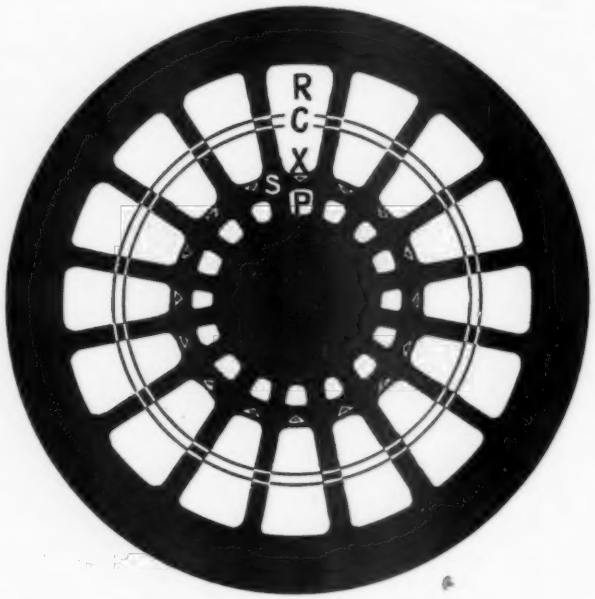



Diagram of structure of a tobacco stem (internode) in cross-section. The black center, black periphery and black radial bands represent the conjunctive tissue, while the white areas represent the general arrangement of the vascular bundles. *R*, outer phloem; *C*, cambium; *X*, xylem (wood); *S*, location of the spiral vessels; *P*, inner phloem. Teratoids may arise by inoculation either from *C* or from the outer face of *P*.

*Xylem.*—Technical name for the wood cylinder. It consists of vessels, wood fibers, and wood-parenchyma traversed by medullary rays. It functions not only as a support and as a carrier (upward) of water and dissolved salts, but acts also as an aerating tissue.

*Cancer.*—In England and France, and in my usage, which follows the common usage, the name for any malignant tumor (hyperplasia) from whatever tissue derived. Among oncologists in Germany and the United States, this term is usually restricted to carcinoma.

*Sarcoma.*—A fleshy, malignant tumor (hyperplasia) produced from connective tissue cells.

*Epithelioma.*—A flat, eroding, malignant tumor (hyperplasia) developed from epithelial cells which grow downward into the subepithelial tissues.

*Carcinoma.*—A malignant tumor (hyperplasia) developed from the epithelium of various glands.

*Embryoma.* *Atypical Teratoid.* *Embryonal Teratoid.* *Solid Cyst.* Terms used to denote the most complex type of tumor in which, in man, the malignant part consists usually of a sarcoma or of a glioma (Askanazy) mixed in with rapidly growing fetal fragments. This form is said to be rather rare in the ovary and rather frequent in the testicle, but occurs in many parts of the body. Said also to be either malignant or not malignant. Probably still confused to some extent with the following:

*Cyst.* *Hollow Cyst.* *Dermoid Cyst.* *Typical Teratoid.* Terms used to designate a non-cancerous tumor consisting of an invaginated surface layer (ectoderm) containing epithelium, hair, fat, and sometimes fragments of various organs, as bones, teeth, etc., or more rarely a very considerable part of the fœtus. The commonest kind is the ovarian dermoid.

## DESCRIPTION OF FIGURES.

FIG. 1.—Tobacco inoculated February 14, 1916, with *Bact. tumefaciens* (hop strain through sunflower in 1915) into leaf axils at the top of the plant when young. *Below:* A rough, brownish, naked crown gall from the middle of which has developed a pale green fused mass of more than 100 leaf-buds. *Above:* At *Y* in the midrib is a non-teratoid naked tumor which has ruptured to the surface. This leaf was deformed from the beginning, *i. e.*, the midrib never had any leaf-blade on the right side. On the under surface of the leaf *X* is a tumor like *Y*, but full of leafy shoots. The tumor at *Z* is also a teratoid. Photo. June 2, 1916. (Natural size.)

FIG. 2.—Tobacco shoot to show size and general appearance of the cut internode (*X*) when ready for inoculation. At *Y* is the leaf axil from which a shoot would have developed. (Natural size.)

FIG. 3.—Similar shoot about 1 month later. This was a check on the inoculations of July 7, and may be taken as a type of the behavior of all the checks on the internodal main axis inoculations described in this paper. A strong sprout (*X*) has pushed from the leaf axil next below the cut, which has healed over normally. Photo. August 9, 1916. (Natural size.)

FIGS. 4, 5, 6, and 7.—A series introduced to illustrate the initial slow start, subsequent rapid progress and equally rapid decay of an internodal main axis (leafy) teratoid tumor on tobacco. Cut internode inoculated by needle-pricks May 31, 1916. Date of photographs: No. 4, June 27. (× 1¾.) Most of the growth here shown had developed during the previous 6 days. No. 5, June 30 (× 4½ *circa*), shows numerous diminutive shoots pushing out of the tumor substance which is growing rapidly. No. 6, July 14, *i. e.*, 17 days after Fig. 4. Tumor now covered with leafy sprouts, a few of which have reached a length of 2 to 3 inches. A normal shoot (*X*) from axil of the leaf *Y*, and corresponding to *X* of Fig. 3, is at the extreme left; all others are from the tumor. Many of the leaves (*Z*) have cleft, one-sided or otherwise defective blades. (× 1½.) No. 7, same as 6 but natural size and from the opposite side where decay has begun (direction of the arrow). There is a small tumor bursting from the top of *X* which is a defective leaf—one lacking most of its blade. At *Y* is another defective leaf—the blade being reduced on the right side.

FIG. 8.—Slow-growing internodal (main axis) leafy tobacco teratoid. All of the shoots are from the tumor and there are no flower-buds. Stem inoculated May 31, 1916. Photo. August 1. (× 1½.)

FIG. 9.—The same tumor as in Fig. 8, but a month later and much larger. Entire time 3 months. Necrosis has begun. All of the small shoots are from the tumor; some are dead and others are dying. The large shoot (*X*) developed from a lower normal leaf axil prior to the formation of the tumor. Photo. August 31. (× 1½.) The last of the teratoids of May 31.

FIG. 10.—Slow-growing internodal crown-gall tumors on tobacco. The upper tumors are teratoids, the hairy masses being small leafy shoots. The lower tumor is a sarcoma. An independent small teratoid has developed at *X*. Inoculated by needle-pricks May 31, 1916. Photo. August 10. (× 8.)

FIG. 11.—Section through Fig. 10. The right-hand (teratoid) tumor originates from the cambium; the left-hand, which is naked and apparently contains no teratoid elements, originates from the xylem side of the cambium (the wood), which is much thickened. The tissues are *t, t*, embryonic (teratoid) elements; *cor*, cortex; *p*, outer phloem; *c*, cambium; *xy*, xylem; *i p*, inner phloem; *m*, pith. (× 8.)

FIG. 12.—Cut surface of two stems of tobacco showing very slow-growing internodal teratoid tumors, the lower dead and the upper dying. They were slow to start and there was no growth in either for a month or six weeks prior to the date of the photograph. The openings are needle-punctures from which no tumors developed, and most, it will be observed, are in the pith. Inoculated May 31. Photo. August 18. (× 4 *circa.*)

FIG. 13.—Internodal crown-gall teratoid on tobacco. More than 50 leafy shoots grew from this tumor. The shoot (*X*) developed after the main axis was removed and before the tumor grew. The influence of the tumor extends not only below in the main axis, but also into the first internode of the big shoot at the left, as shown by the budding roots (*R, R*), absent above and below. Inoculated May 31. Photo. June 25. (× 1½ *circa.*)

FIG. 14.—Top view of the tumor shown in Fig. 13. No flower-buds but numerous green (leafy) sprouts out of the pale brown naked substance of the tumor. (× 3 *circa.*)

FIG. 15.—Slow-growing crown-gall tumors on cut surface of tobacco stem (internode). Numerous small sarcomas and also five distinct centers of (leafy) teratoid growth, four of which (*t*) have originated on the inner edge of the vascular ring. The teratoid (*X*) near the surface is probably from the cambium. The teratoid tissue, of course, is covered with an epidermis; the sarcomas are naked. For a longitudinal section in direction of the arrows see Fig. 17. Photo. June 26. (× 7.)

FIG. 16.—Crown-gall tumors on cut surface of tobacco internode. Several, small, naked tumors and at least three distinct centers of teratoid growth which are from the inside of the vascular ring. The roundish openings are needle-stabs from which no tumors developed. Stem inoculated May 31, 1916. Photo. July 3, 1916. (× 12 *circa.*)

FIG. 17.—(a) Longitudinal section of Fig. 15 showing teratoids (*t, t*) arising inside the cambium line (*c, c*).

(b) Cross-section of a normal portion of the same stem. *Cor*, cortex; *v*, vascular ring; *m*, medulla; *o p*, outer phloem; *i p*, inner phloem. Planar enlargement from fresh material.

FIG. 18.—Longitudinal section of a very slow-growing crown-gall teratoid (leafy) tumor on cut surface of tobacco internode (right side). The growth is from the cambium and the wood is thickened. The cortex is not involved. On the left side (top in figure) is a cortex tumor. This does not involve the cambium unless sections are missing from the series, yet it contains some teratoid elements. It is probably from the cambium because the cambium and wood under it are sarcomatous and at another level the cross-cut (inoculated) surface of the stem (at *X*) bears a third small tumor which is a teratoid and originates in the cambium. Inoculated May 31, 1916. Collected and fixed August 18. Slide 1271-13. (Planar enlargement.)

FIG. 19.—Crown-gall teratoid on tobacco, showing flower buds. The shoot at the right (*X*) grew from the leaf axil bud below prior to the development of the tumor. The tumor at this date bore numerous green leaves and five flower buds, and the base of the

shoot at *Y* was swollen. For the appearance of this tumor nine days later when it was nine times as large, see Fig. 20. Plant inoculated July 7, 1916, on the cut surface of the main axis (internode) near the top of the plant which had not yet blossomed, but was nearly ready to develop flower buds. Photo. July 31. (Natural size.)

Fig. 20.—Same as Fig. 19. Several of the earliest flower buds, which developed stamens and pistils but never opened, have now fallen off, as at *X*. The position corresponds (nearly) to that of Fig. 19, made nine days earlier, but the tumor is much larger and many new teratoid elements have developed. All the remaining flower buds (*b*, *b*, *b*) fell off a few days later. Fasciation at *f* and in other places. Photo. August 9. (Nearly natural size.) There was at this time no necrosis, but decay set in a few days later, and on September 1 all of the teratoid elements were either dead or dying, and the body of the tumor was beginning to shrivel.

Fig. 21.—Crown galls on the cut stem (internode) of a tobacco plant. The inoculations were made July 7, 1916, into the cortex. A few needle-thrusts may have entered the cambium as at *X*, *X*, but none of the tumors has developed shoots, *i. e.*, they are naked sarcomas. Nearly every needle-thrust produced a tumor. The two larger tumors at the top were of the same size as the others 48 hours earlier. Photo. July 31. (× 8, nearly.)

Fig. 22.—Teratoid crown gall on tobacco. The main axis was cut off at the top of the plant in the middle of an internode, and the cut end inoculated on July 7 by means of needle-pricks from an agar streak culture of *Bact. tumefaciens; X* and *Y* are normal shoots which developed after the top was removed and before the tumor appeared, the top of *Y* being cut away at the time of making the photograph; *Z*, *Z'*, are the subtending normal leaves. Tumor filled with shoots, the bases of many of which are swollen (sarcomatous). Photo. August 4. (Nearly natural size.) No necrosis.

Fig. 23.—Same as in Fig. 22, but 10 days later (38 days from date of inoculation) when wilting and shriveling of the teratoid shoots had begun, and also a decay of the body of the tumor in several places, as at *a*, *b*, where a white fungus has appeared (Fusarium sp.). Fasciations occur and all of the shoots are from the body of the tumor. No flower buds appeared. Photo. August 14. (Natural size.)

Fig. 24.—Crown-gall teratoid developed from the middle of a tobacco (main axis) internode. Shoots *X* and *Y* began to grow as soon as the top was removed, *i. e.*, in advance of the tumor; *Y* is normal, *X* has become infected with internal tumors (the raised light spots) which are invasions from the primary tumor. Tumor filled with luxuriant leafy shoots. Plant inoculated on the cut surface of the internode July 7, by needle-pricks. Photo. August 1. (Natural size, nearly.)

Fig. 25.—Same as Fig. 24, but 14 days later (38 days from inoculation). The tumor has developed rapidly and in the upper part are now to be seen a multitude of closely packed pale green leaf-buds like those shown in the middle of Fig. 1. No evidence of flower buds. The larger shoots have begun to shrivel, but there is as yet no necrosis. The tumors in the big branch at the left have not ruptured to the surface. Photo. August 14. (× 1⅓.)

Fig. 26.—Same as in Fig. 25, but 23 days later (2 months after inoculation) when all the shoots have shriveled and necrosis of the body of the tumor is far advanced. Photo. September 6, 1916. (Natural size, nearly.) For a view of the opposite side of this tumor on the same date but one quarter natural size, see lower tumor of Fig. 29.

Fig. 27.—*a*, *b*. Top view of two crown-gall teratoids produced on cut surface of tobacco internodes. The tumors contain many distinct centers of greenish (leafy) teratoid growth, but none of them has passed much beyond the stage of buds, or judging from other similar tumors would have done so later. Tumors not yet beginning to decay. Stems inoculated July 7, 1916. Photo. July 26. (× 3.)

Fig. 28.—Crown-gall (main axis) tobacco teratoids. All were produced from the middle of cut internodes by needle-prick inoculations. Introduced to show rapid decay and varying types of the tumor. Nos. I-IV are alike, whereas No. V contains a mixture of large and small shoots. No. VI is the fleshy back part of No. V. The upper row represents plants inoculated July 7, and the only living tumor parts are a small portion of the top of III at *X*. The lower two stems were inoculated July 29, and the tumors were still free from decay. The left-hand one contained many distinct centers of teratoid growth, *i. e.*, several hundred. For enlargement of one lobe of a similar tumor see Fig. 30. Photo. September 13, 1916. (⅔ natural size.)

Fig. 29.—Three crown-gall teratoids on tobacco internodes. The lower tumor, far advanced in decay and covered with mold (Fusarium sp.), is the one already shown in Fig. 26. The upper tumors are internodal inoculations of July 29, 1916. All of the leafy shoots are from the tumors. Some are swollen and distorted. No flower buds were observed and there was as yet no necrosis. Photo. September 6, 1916. (5/16 natural size.)

Fig. 30.—One lobe of an internodal (main axis) tobacco crown-gall teratoid on which there were no well-developed shoots, but 469 diminutive pale green buds, all of which were like those on the lobe here shown. Query: Did all grow from displaced "germ cells" or from normal somatic cells exposed to a special stimulus? Inoculated July 29, 1916, on cut surface by needle-pricks. Photo. Sept. 13. (× 7, nearly), with Zeiss 100 mm. planar. Necrosis not begun. Material fixed for sections. See Fig. 31.

Fig. 31.—Two-thirds of a cross-section of Fig. 30 in which are 30 centers of embryonic growth. The central pale, coarse-celled tissue is cortex; the dark-stained, irregular (stringy) central tissue is the sarcoma. Methyl green and acid fuchsin stain. (× 18.)

Fig. 32.—Tobacco crown galls 46 days old. The plants were inoculated on the cut surface of the main axis in the middle of internodes on July 29, at which time and subsequently they seemed to be equally good plants. They were standing side by side and developed equally vigorous tops from the side shoots *X*, *X*. No. *a* is a sarcoma and behind it (unseen) are two other much smaller tumors of the same sort. No. *b* is a leafy teratoid, the shoots of which are beginning to wither. The leaves have wilted naturally, not as a result of cutting the stem. The bulk of this tumor is 1500 times that of *a*. Photo. September 13. (About natural size.)

Fig. 33.—South end of one of our experimental houses showing part of tobacco plants used for Series V. Photo. Sept. 19, 1916, *i. e.*, 12 days after the inoculation, at which time vigorous side shoots were pushing, but no tumors had appeared. (Greatly reduced.) On a bench in the background are the inoculated balsams.

Fig. 34.—Tobacco crown-gall teratoid. One of the inoculated stems of Fig. 33 at the end of nine weeks showing one small leafy tumor (*X*) emerging well within the vascular ring. (× 4.)

Fig. 35.—Section of tobacco crown-gall teratoid (Fig. 34) showing origin of the tumor chiefly from the outer face of the inner phloem—cortex not involved. The lifted up portion (at *X*) is cork which formed after the stem was cut and before the tumor appeared. The wings of the arrow-shaped growth are leaves in which the palisade tissue is reversed, *i. e.*, it is below facing the axis of support. Block 1295-41. (Planar, 50 mm.) The part under *x* is sarcoma; all to the left of *x* is teratoid.

Fig. 36.—Middle (sarcomatous) part of Fig. 35 below the surface at *X*. The two small right-hand lobes of the tumor have arisen from the cambium, the remainder of the tumor is from the inner phloem region. Special attention is called to the loss of polarity on the part of these cells. For an enlargement of the part designated by the arrow see Fig. 38. (× 75.)

Fig. 37.—Photomicrograph from Fig. 35 but under 36, showing continuation of the sarcomatous inner phloem (the darker stained part), *i. e.*, the bottom of Fig. 36 is the same as the top of Fig. 37. The tissues are: *m*, pith; *ph*, abnormal inner phloem; *sp*, spiral region of the bundle; *tr*, tracheæ; *c*, cambium; *sv*, outer phloem; *cor*, cortex; *s*, sarcomatous cambium. (× 75.)

FIG. 38.—A detail from the middle of Fig. 36 (in direction of the arrow), showing loss of polarity in the sarcomatous cells. Pith cells (*m*) at the left; *tr*, abnormal vessels (tracheæ) developed in the tumor; *sp*, normal spiral vessels of the stem lying undisturbed at the inner border of the xylem. Section stained with acid fuchsin and methyl green. (× 160.)

FIG. 39.—Slow-growing (main axis) tobacco crown-gall teratoids. Plant inoculated by needle-pricks in the middle of the cut internode on September 7, 1916. Here apparently are two tumors fused, one from the ordinary cambium, the other from a meristem developed from the outer face of the inner phloem. See Figs. 40 and 41. Photo. November 15, 1916. (× 1¾.)

FIG. 40.—Section through the middle part of Fig. 39, showing origin of the bulk of the tumor from the cambium. The parts are: *cor*, cortex; *p*, outer phloem; *c*, cambium; *tr*, xylem; *i p*, inner phloem; *m*, pith; *x*, level of Figs. 42 and 43. The middle part of the tumor (*s*) is sarcomatous; the outer parts (*t, t, t*) with cells arranged normally are the teratoid portions. At *i p*, there is a small tumor capping the inner phloem (see Fig. 41). Slide 1302-6. Planar, 50 mm. (× 10.)

FIG. 41.—Section of Fig. 39 at one edge (slide 1302-36) showing two distinct tumors (both teratoid), one (*c*) from the cambium, the other (*i p*) from the region of the protoxylem and inner phloem. The darker parts of the larger tumor (*s, s, s*) are sarcomatous and the paler parts (*t, t, t, t*) teratoid. The interrupted dark stripes below the tumor (along the inner edge of the vascular bundle) represent the sarcomatous inner phloem, a small portion of which at the level of *X* is shown enlarged in Fig. 42.

FIG. 42.—Protoxylem region between Figs. 40 and 41 at *X*, enlarged to show changed appearance of that part of the vascular bundle. Unchanged spiral vessels (*s*) at left, to the right of which are two strands of inner phloem containing tracheæ (*tr.*) and distorted parenchyma cells (companion cells?). The body of the wood lies at the left of *s*. The large pale cells (*m, m*) are pith cells. At *st* are sieve tubes. Slide 1302-12. (× 160.)

FIG. 43.—Right (innermost) phloem strand of Fig. 40 at *X*, enlarged, showing sieve tubes in the center (*st*) and well developed tracheæ on both the inner and the outer face of the strand, *i. e.*, tissues which occur normally only in the wood at the left of the spiral vessels, together with plain evidence of sarcomatous invasion as shown by multiplication and distortion of short cells. This is virtually a concentric bundle with phloem at the center. At either side are the cells of the pith (*m, m*). (× 210 *circa.*)

FIG. 44.—Tobacco crown-gall teratoid from a main axis internodal (cambium) inoculation of September 7, 1916. At *X* there was a sprout of the same size as *Y*, which fell off in handling. Masses of green buds at *Z*. Photo. January 10, 1917. (4/5ths natural size.) Back view.

FIG. 45.—Front view of Fig. 44 slightly enlarged. Tumor very slow growing and first visible as an internal swelling (*X*) a centimeter below the cut surface (see text). Necrosis and sloughing at *Y*. Time 125 days.

FIG. 46.—*a*. Tobacco crown-gall teratoid. Main axis internodal (protoxylem) inoculation of September 7, 1916. Collected November 14, 1916. Very slow-growing tumor. (Photographed under alcohol; × 3.)

*b*. Slow-growing teratoid from same series (Fig. 33) as *a*, but a cambium inoculation. This is the largest tumor obtained from the inoculations of September 7. Photo. January 25, 1917. (× 2½.)

*c*. Two small, slow-growing tumors from same series as *b* (cambium inoculations of September 7). Both are teratoids and no other tumors developed on the cut surface, although the lower received 16 needle-pricks and the upper nearly as many. Time 124 days. (× 8.)

FIG. 47.—Slow-growing teratoid crown gall from inoculations of September 7, 1916. Fixed December 6. Cortex at right. The tumor originates from the region of the inner phloem (see Fig. 48). Doubtful where inoculated (inoculation said to have been into the cortex). Slide 1305-15, near margin of tumor. (× 17.)

FIG. 48.—Same as 47, but a more central cut showing clearly the origin of the tumor from outer face of inner phloem (*i p*); the cambium and cortex are not involved. The tissues are: *m*, normal pith; *i p*, diseased inner phloem containing tracheæ and sarcomatous cells; *sp*, normal spiral vessels of the stem bundle; *tr*, tracheæ; *c*, cambium; *o p*, outer phloem; *cor*, normal cortex; *t, t*, teratoid elements of the tumor; trichome at *h*. (Planar, 50 mm. Slide 1305-11, × 70.)

FIG. 49.—North end (about ½) of two rows of out-of-door tobacco plants used for Series I and II of August 1 and 5. Photo. Sept. 19, 1916. Pathological houses in the background.

FIG. 50.—Whole crop (nearly) of successful inoculations of August 1 and 5 (Series I and II, out-of-door) with most of the failures. The right five of the lower row are inoculations of August 1. The remainder are those of August 5. At *X, X* are small tumors bursting out below. The first four tumors of the top row are teratoids and at *A* is one originating from the region of the inner phloem as serial sections have shown clearly. Introduced for comparison with indoor inoculations of July 29 (Figs. 28, 29, 30, and 32). The ends of all these stems, when photographed, were living but rather firm. The same cultures were used on the last five of the lower row as on the Pelargoniums shown in Figs. 60, and 61. Photo. September 19, 1916, slightly enlarged.

FIG. 51.—Section of tobacco crown-gall teratoid at *A* in Fig. 50, showing origin of the tumor from the inner face of the vascular bundle. The tissues are: *s, s*, sarcomatous elements; *t*, teratoid portion; *cor*, cortex. Slide 1286B-14. (× 23.)

FIG. 52.—Pelargonium crown-gall teratoid at the end of four months. Inoculated into a leaf axil January 13, 1916, from a 3-day-agar culture of the hop strain of *Bact. tumefaciens*. The shoots from the tumor were dying or dead, *i. e.*, greenish yellow or brown. The back side of the tumor was also full of shoots. The main axis above is bent and dwarfed and has dropped four of its leaves, while several others (the lighter colored ones) are pale green or yellowing. All the leaves below have fallen except the reflexed dying one at the left. Photo. May 16, 1916. (5/7ths natural size.)

FIG. 53.—Pelargonium crown-gall teratoid—an internodal inoculation which has covered the whole top of the cut stem. The inoculation like all the others was made by needle-pricks without hypodermic injection. The tumor contains four distinct centers of green leafy growth. These parts are covered with an epidermis and are hairy, while the remainder of the tumor is naked. Inoculated July 7, 1916. Photo. August 1, 1916 (straight down). (× 7 *circa.*)

FIG. 54.—Pelargonium internodal crown-gall teratoids. Inoculated on the cut surface July 7, 1916. (a) The whole top of the stem is covered by the tumor. There is fasciation, *i. e.*, the blade of the upper leaf is fused into a conical cup. All the shoots are living. Photo. August 9, 1916. (× 1⅓.) (b) Another plant showing numerous leaves growing from the tumor, the larger of which have begun to wither. Two small leaves which interfered with the view were cut away from the first node below. Photo. August 24. (Natural size.)

FIG. 55.—Cut surface of Pelargonium stem inoculated July 7 and photographed August 16, 1916. The slow-growing tumors are all sarcomas, at least, no teratoid elements are visible on the surface. (× 6 *circa.*)

FIG. 56.—Pelargonium internodal crown-gall teratoid. Showing whole top covered by the tumor and petiole at the left (*f*) fasciated. All parts living. Inoculated on cut surface July 7, 1916. Photo. August 9, 1916. (× 1¾ *circa.*)

FIG. 57.—Pelargonium internodal crown-gall tumors, sarcomatous and mixed (teratoid). Very slow growing. Cut end of stem

inoculated July 7, 1916. Photo. August 15, 1916. (× 7.) Side view. Teratoids at the top.

Fig. 58.—Same as Fig. 57, but a top view. The tumors at the bottom are naked and without shoots. For a vertical section see Fig. 59. (× 7 *circa*.)

Fig. 59.—Section of Fig. 57. Block 1267, Slide 42. At the top are the non-teratoid tumors which have developed (for the most part, at least) from the cortex. At the bottom is the teratoid which does not, or did not at first, involve the cortex, but is an outgrowth of the cambium. (× 12, nearly.)

Figs. 60, 61.—Pelargonium crown-gall teratoids. All were inoculated August 1, 1916, on the cut end of internodes, *i. e.*, remote from buds. Each one of the 16 tumors contained teratoid elements. All but No. VIII were alive when photographed. Nos. II, III, and XI show closely set masses of buds. No. II furnished the section shown in Fig. 80. No. XVI at *X* shows twisting and fasciation of the petiole which bears two leaf blades. Two petioles are also fused at *X* on No. VI. Photo. on a W. and W. panchromatic plate September 13, some of them end on. Slightly enlarged.

Fig. 62.—Pelargonium crown galls in the middle of a leaf-stalk. The lower tumor bears a cluster of young leaves. Petiole inoculated by needle-pricks with *Bact. tumefaciens*. September 29. Photo. December 6, 1916. (× 3.)

Fig. 63.—Pelargonium teratoid tumors originating naturally at *X* and *Y* on gardener's cuttings bedded in earth for propagation. The specimens came from a gardener's house near Baltimore. Both show fasciation (as at *f*). Roots (*R*) as well as shoots are growing out of the left tumor on II. Although not visible in the photograph, there is a flat tumor at *Y* in the base of II. Photo. March 18, 1917. (× 1⅜.)

Fig. 64.—Axillary crown-gall teratoids on Ricinus. Inoculated by needle-pricks in two upper leaf axils March 17, 1916, with hop strain of *Bact. tumefaciens* passed through sunflower in 1915. Axis twisted; top (direction of the arrow) aborted; subtending leaves reflexed; sprouts from the tumors. The petiole is twisted swollen (sarcomatous) and a tumor has ruptured from its interior at *X*. This leaf collapsed a day or two later. The smoother parts of the tumors are covered with a membrane. Photo. April 29, 1916. (Natural size.)

Fig. 65.—Axillary crown-gall teratoids on Ricinus. Inoculated by needle-pricks into two upper leaf axils March 17, 1916. Stem leaves reflexed and withering. Top at *X* aborted. The teratoid shoot at *Y* has developed a small tumor. The subtending petiole at *Z* also bears a tumor. Both of these were at first interior growths. *L* is a dead leaf subtending the lower tumor. Photo. April 29, 1916. (Natural size.) Three days later the plant died.

Fig. 66.—Axillary crown-gall teratoids on Ricinus. Inoculated by needle-pricks into two upper leaf axils, March 17, 1916, with hop strain of *Bact. tumefaciens*. Main axis dwarfed and distorted but growing point not destroyed as in 64 and 65, subtending leaves reflexed (the lower one, at *C*, has fallen and the other, at *P*, is dead). The upper tumor has involved all of the internode up to the third leaf (*pl*) which is yellowing. At *W* is a mass of green buds and fused leafy shoots. Tumors encapsulated at first. Afterwards the sarcoma ruptured to the surface as at *X*, *Y*, *Z*. Photo. April 29, 1916. (× 1¼.)

Fig. 67.—Axillary crown-gall teratoids on Ricinus. Left plant inoculated March 21, into two upper leaf axils. The tumors have grown rapidly and are partly naked. The main axis has aborted and the subtending leaves are reflexed. The plant is very badly dwarfed and ready to die. The control plant is on the right. On the date of inoculation the two plants were of the same size and equally vigorous. Photo. (much reduced) April 29, 1916. Time 39 days.

Fig. 68.—Cross-section of tumor strand in petiole of Ricinus—the kind of strand that yielded only sarcomas. Inoculated by needle-pricks into the blade of the leaf March 21, 1916. Collected and fixed May 19. Cross-section in middle of petiole 9 cm. below the blade of the leaf which bore many tumors. Wood (*xy*) above, pith (*M*) below. This tumor strand (*S*) gave rise to hummocky swellings at frequent intervals and all that side of the petiole was thickened, especially the wood, but none of the tumors had ruptured to the surface. Slide 1225-A-18. (× 52.)

Fig. 69.—An enlargement of the center of Fig. 68, showing in greater detail the interior and the surroundings of the tumor strand. A sort of fibrous tissue (thickened bundle sheath?) surrounds it. This stains very much like the collenchyma. (× 200.)

Fig. 70.—Encysted, slow-growing, crown-gall tumors on okra (*Hibiscus esculentus*). Plants inoculated June 15, 1916, by needle-pricks into leaf axils with the hop strain of *Bact. tumefaciens*. By "encysted" I mean that the right-hand tumor is entirely covered by cortex and cork, and the left-hand one also except in four places (*X*, *Y*, *Z*, and one place on the back) where the nodular naked sarcoma has broken through. The right tumor is sound. The left one is beginning to decay in its older part (*X*). No. I is a teratoid and with a hand-lens a single small sprout can be seen just below *Y*. No. II was also inoculated into a leaf axil, but it gives no external evidence of being a teratoid. At *W*, a ripe fruit was cut away. Photo. September 22, 1916. (⅘ths natural size.)

Fig. 71.—Same as I of Fig. 70, but from the back side. The lettering is the same. *Z* is a naked, rapidly proliferating, sarcomatous mass, but *C* is cortex covered by a cork layer. There is also a small naked portion behind the branch *B*. Photo. September 22, 1916. (× 2, nearly.)

Fig. 72.—Cross-section of an unruptured okra tumor from same series as Fig. 70, showing that the bulk of the bacterial energy has been expended on the wood and on the pith, which is full of distorted fibro-vascular elements. The cells of the greatly enlarged cortex are normally arranged and not sarcomatous. The black spots in the cortex are deep-staining mucilage glands. The tissues are: *m*, pith; *xy*, wood cylinder; *ph*, phloem; *cor*, cortex; *c*, cork layer; *camb*, cambium line; *tr*, tracheæ and other tissues which are inclined at a right angle to the general direction of the vessels of the wood. (× 10 *circa*.)

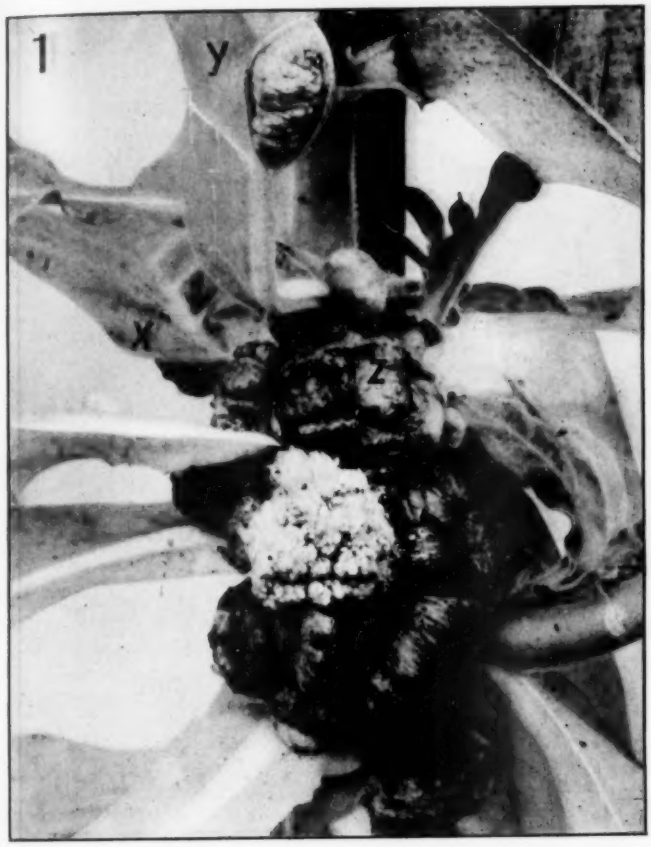
Fig. 73.—Detail from Fig. 72 at *X*, showing modified pith-cells traversed by distorted fibers and vessels. (× 68.)

Fig. 74.—Axillary crown-gall teratoids on the common garden balsam (*Impatiens Balsamina*). Inoculated July 26, 1916, with the hop strain of *Bact. tumefaciens* in the leaf axils prior to the appearance of branches. Plant just beginning to blossom. The tumors contain roots (*R*), sarcomatous leafy shoots (*X*) and flower stuff. Photo. August 15, 1916 (natural size, on a W. and W. panchromatic plate with a red screen. The stems were actually pale green and the tumors red).

Fig. 75.—Same series as in Fig. 74, but farther along in development. The top is dwarfed and diseased branches have developed. The tumors are red (with flower stuff), roots are growing from them freely, and in two places (*X*, *Z*) small leafy shoots are visible. The distorted leaf *Y* also shows internal and ruptured tumors. Inoculated July 26, 1916. Photo. September 19, 1916. (Natural size.) The top of the plant extends only 2 inches above the part here shown and the diameter of the stem immediately beyond the tumor is only ⅛ inch.

Fig. 76.—Leaf axil crown-gall teratoids on the garden nasturtium (Tropæolum). Plants inoculated August 5, 1916 (hop strain through sunflower in 1915, colony 1). The tumors developed both leafy shoots (*X*) and roots (*Y*, *Z*), but they are now decaying. Photo. November 13, 1916. (Nearly natural size.)

Fig. 77.—Leaf axil crown-gall teratoids on cabbage. Inoculated by needle-pricks September 29, 1916, with the hop strain of *Bact. tumefaciens*. The inner edge of the left tumor is smooth (*membranous*) and bears a leafy shoot. The rest of this tumor, and the whole of the right tumor, is naked and sarcomatous. Leaf scars are visible on the stem. Photo. January 4, 1917. (× 1¾ *circa*.)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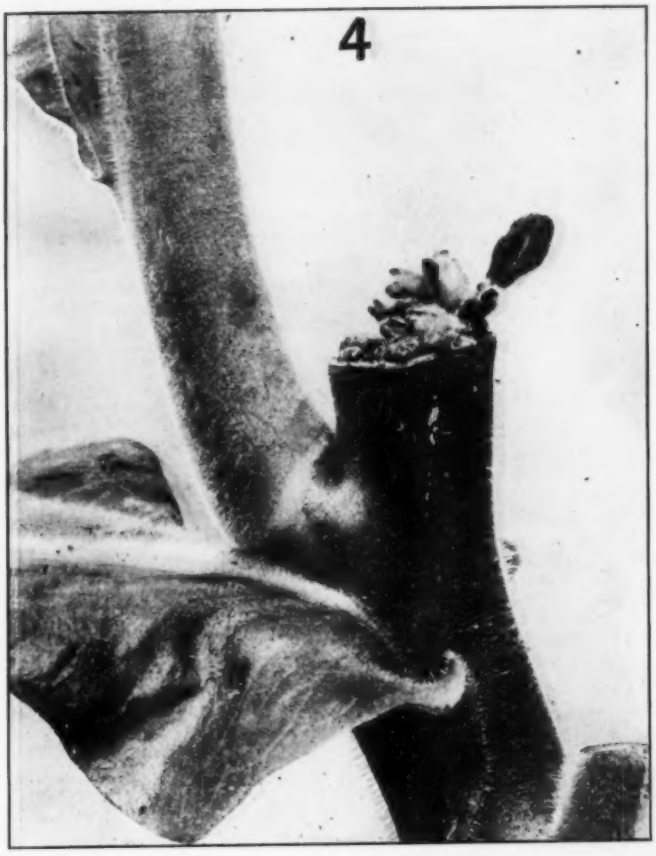
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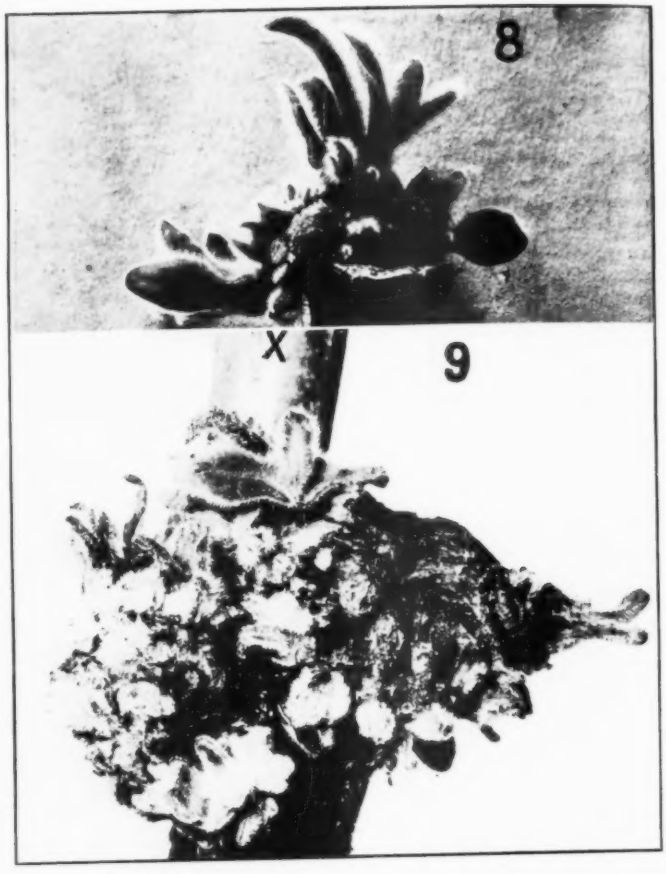
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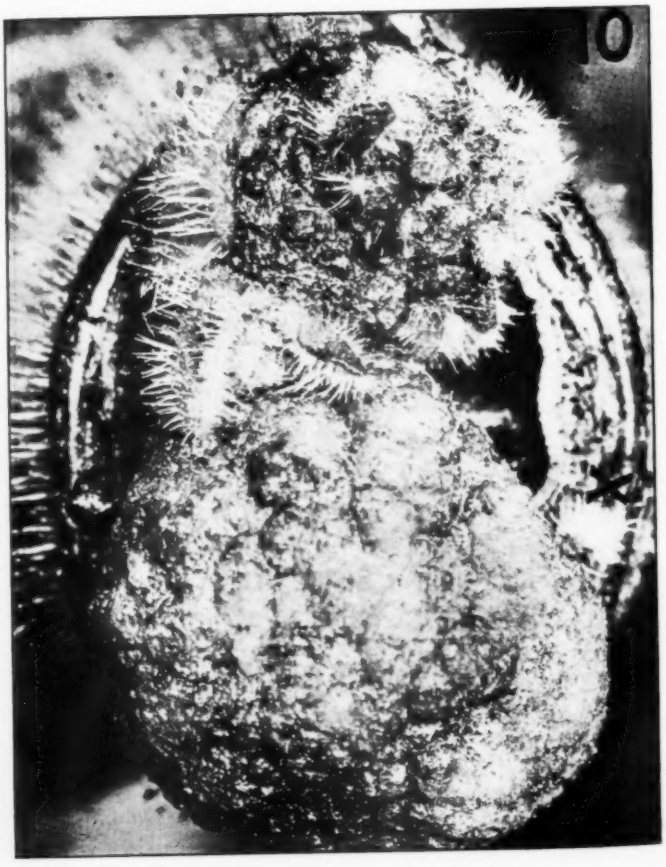
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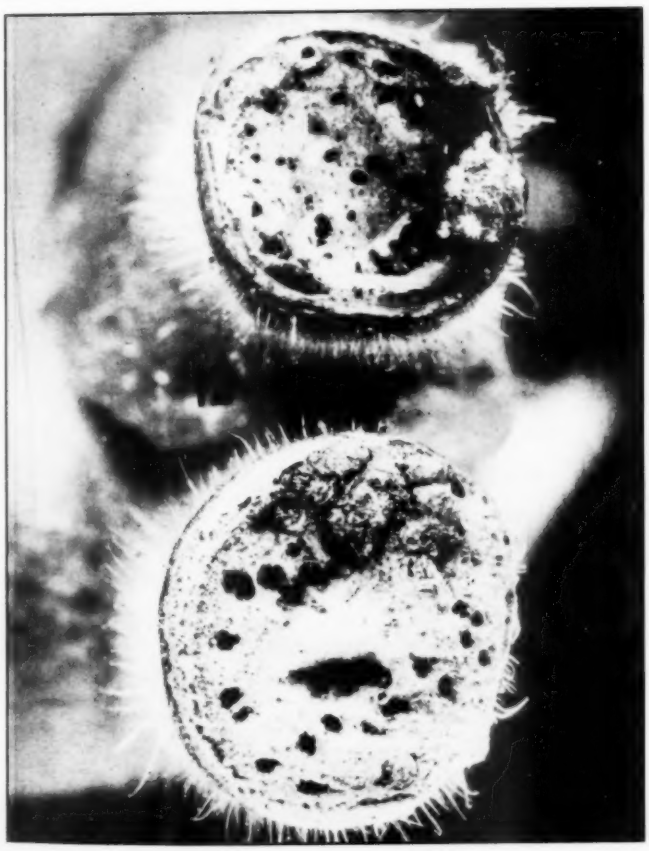

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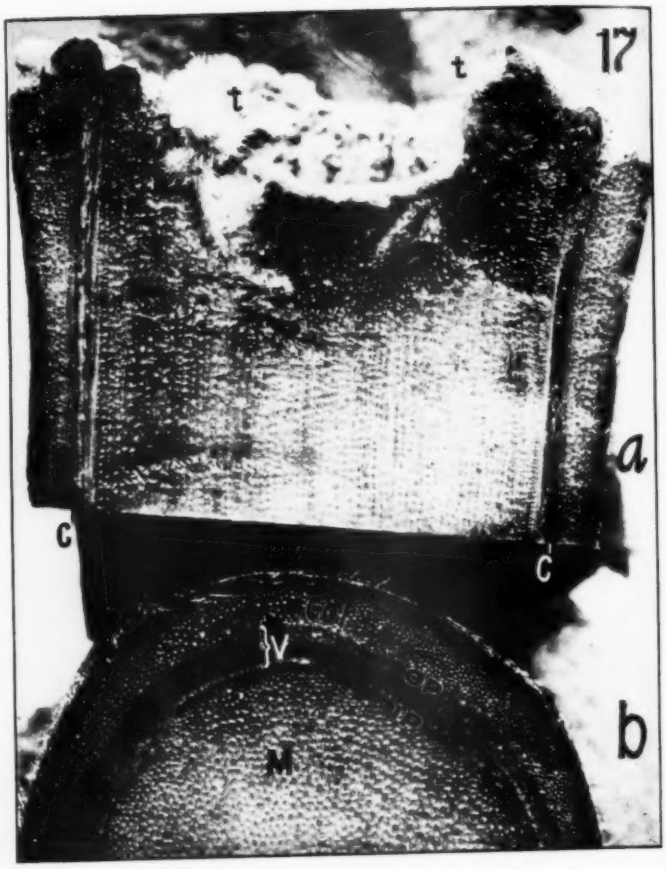
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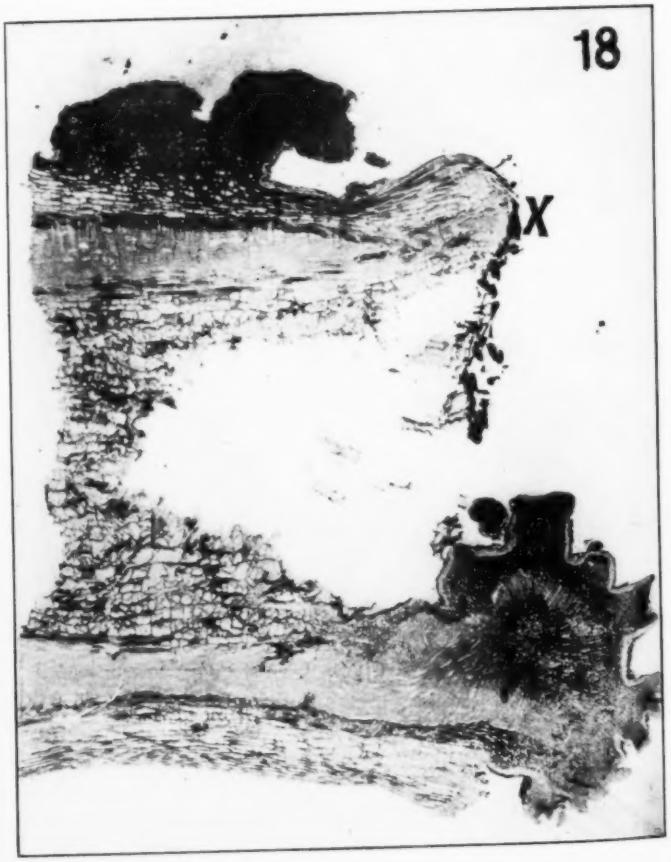
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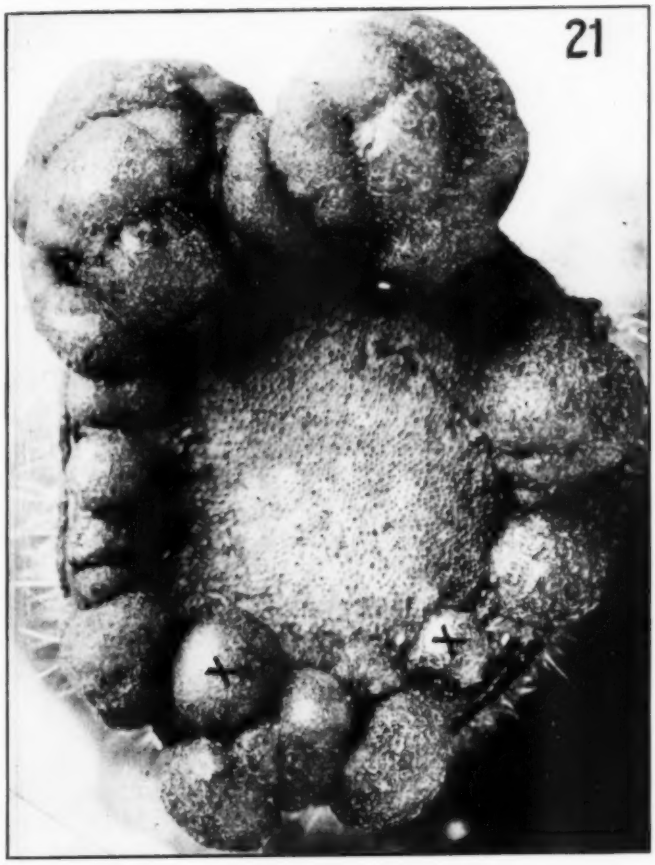
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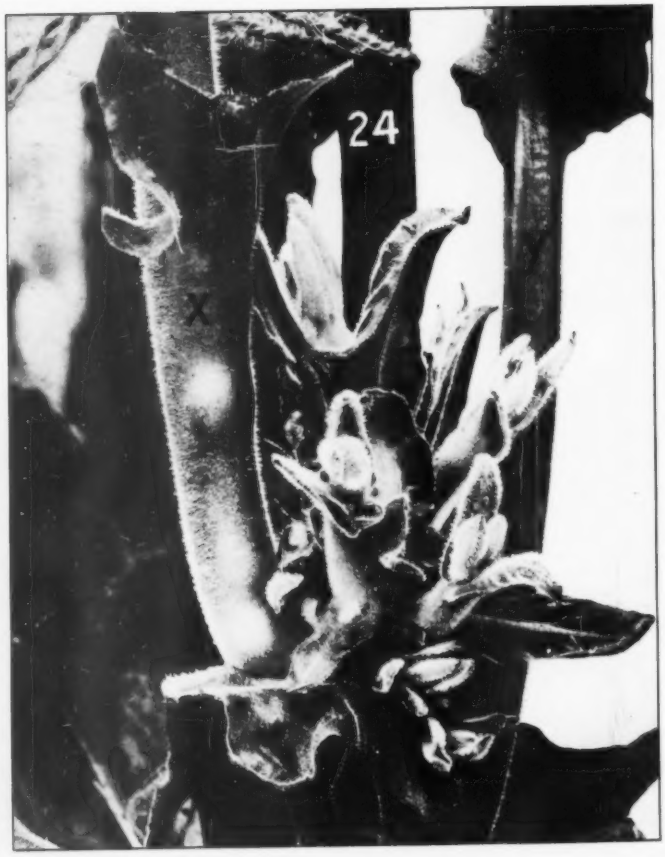
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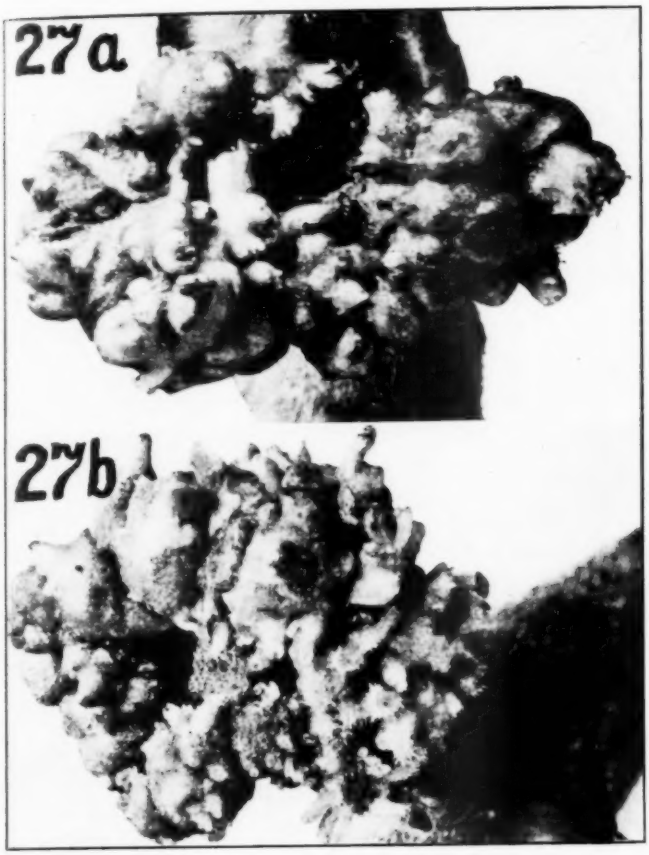
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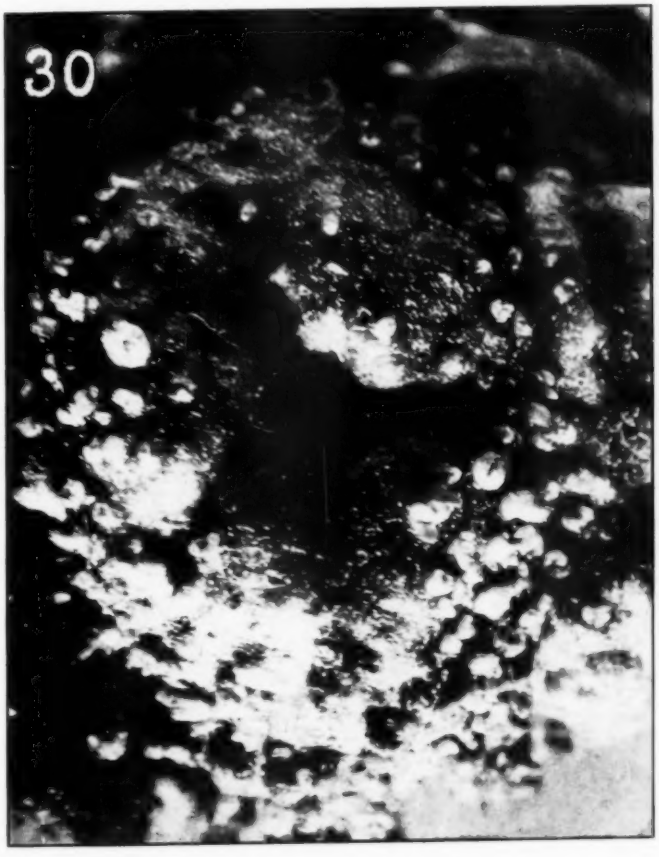
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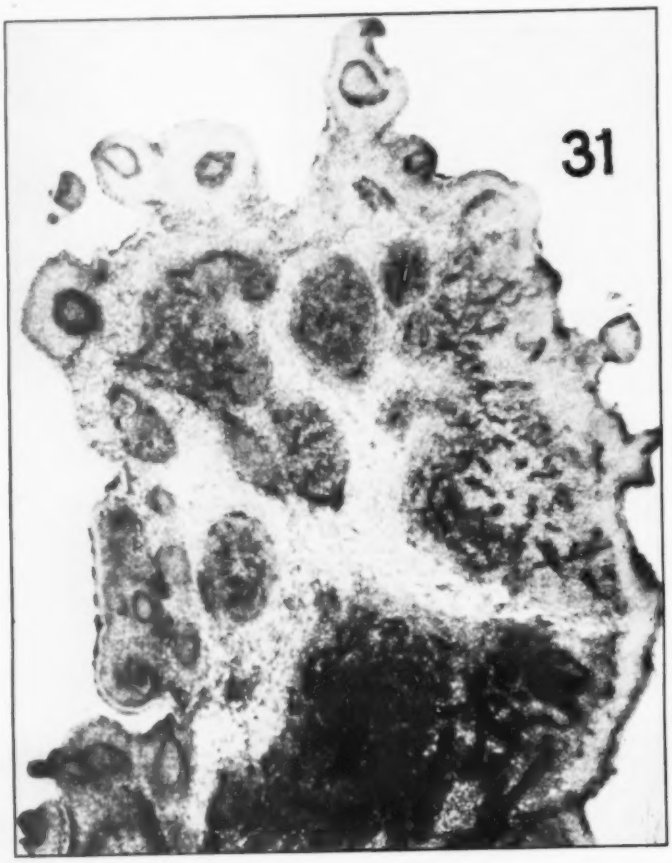
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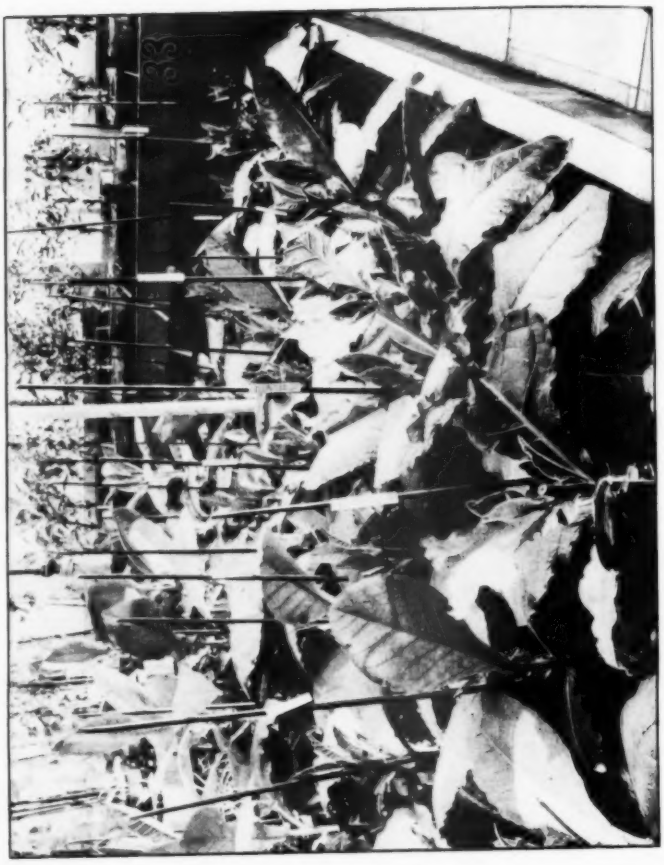

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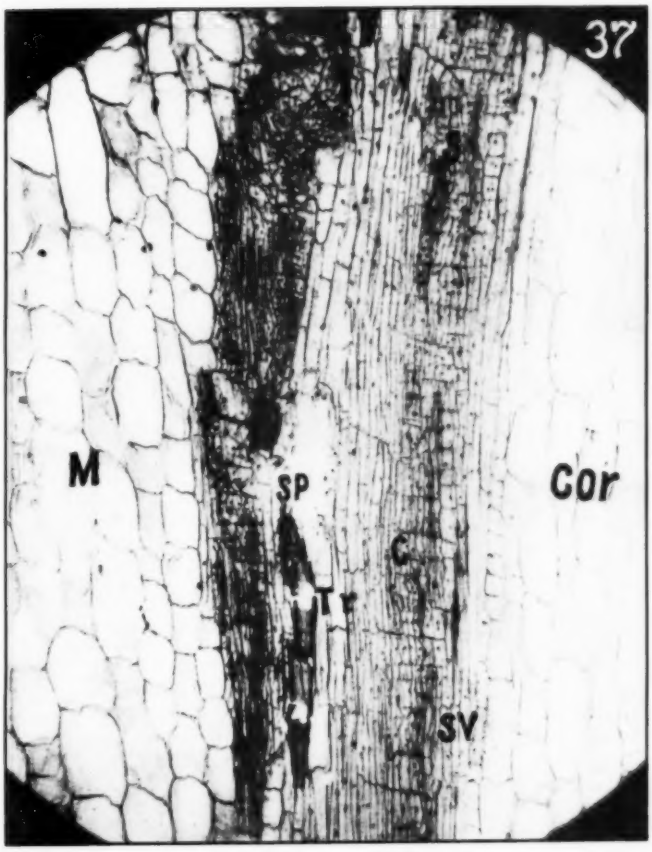
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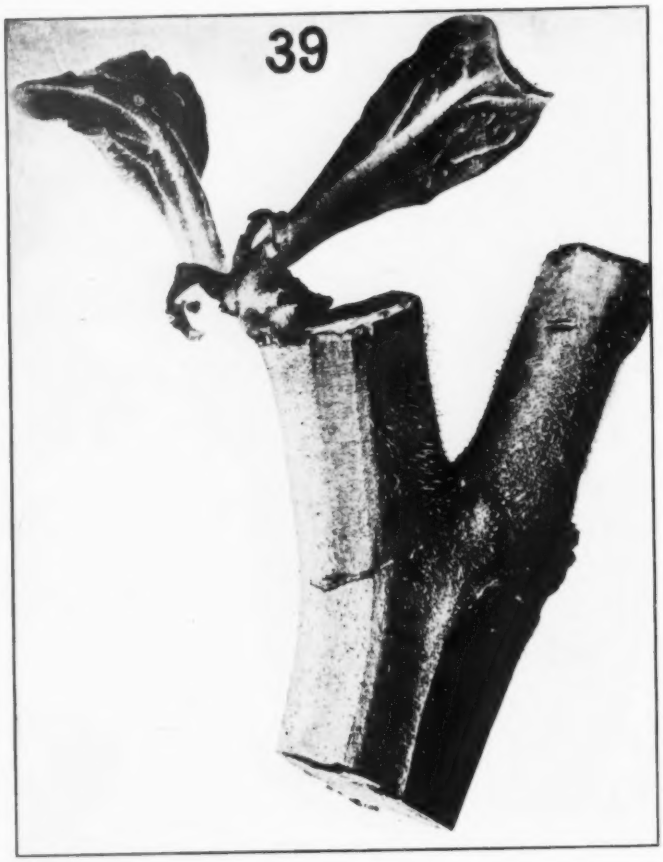
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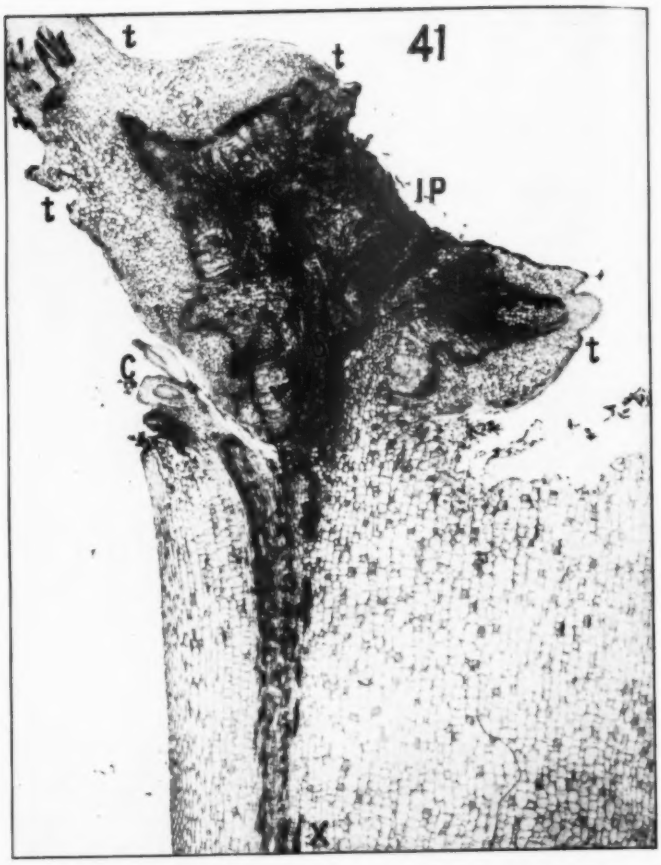
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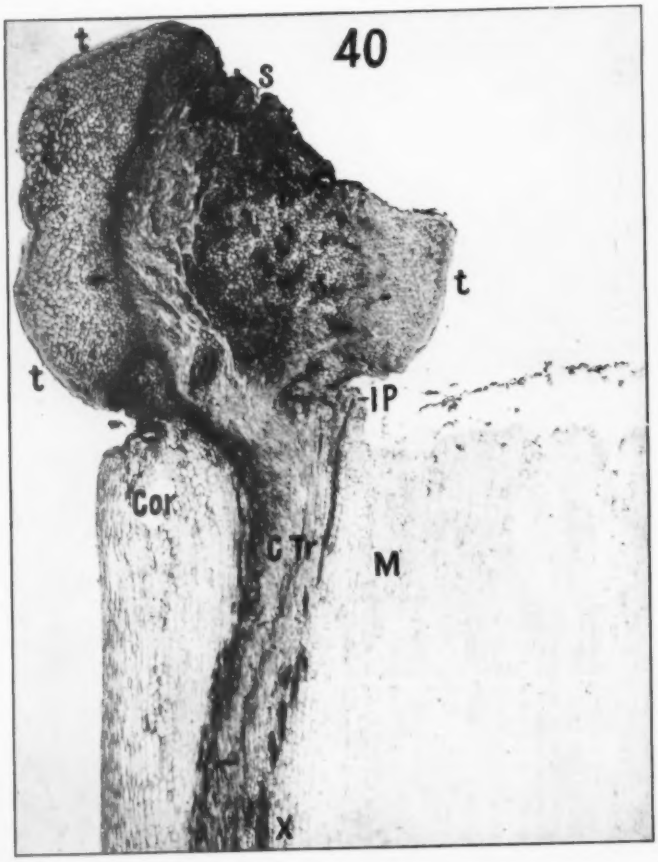
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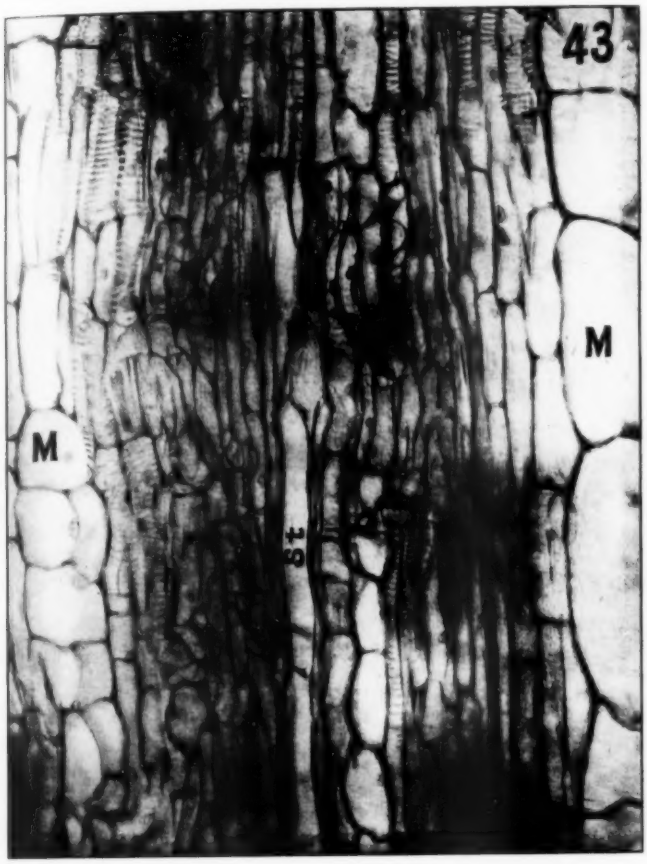
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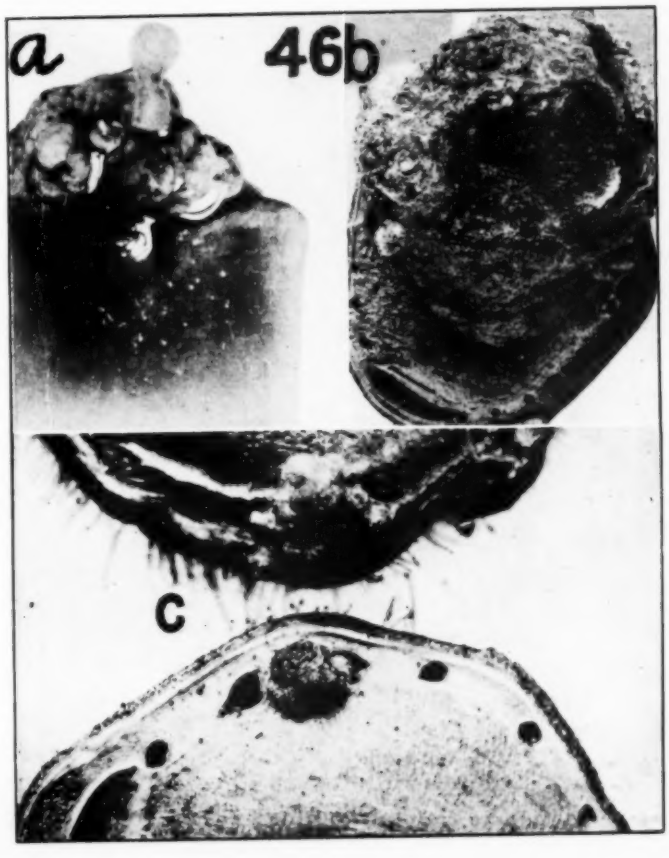
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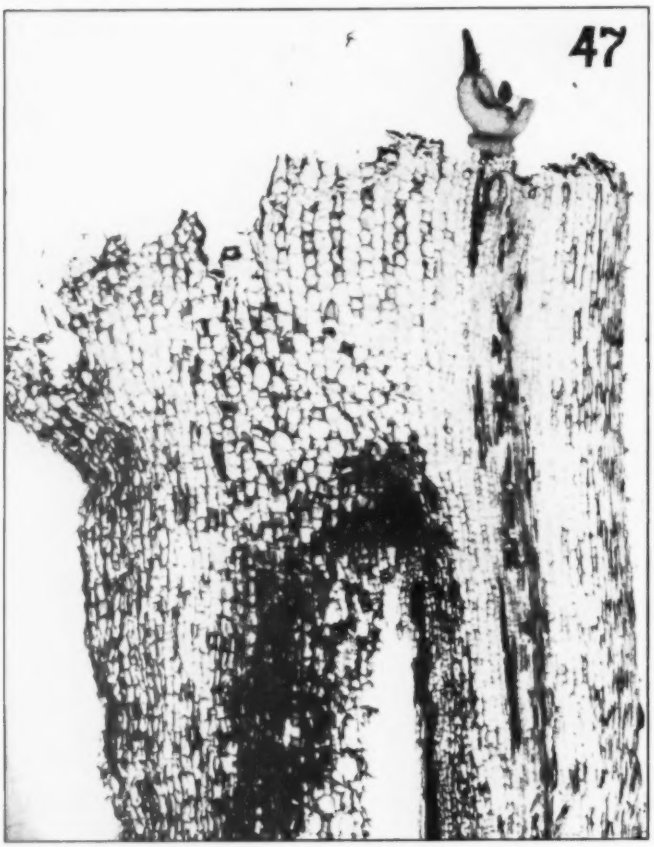
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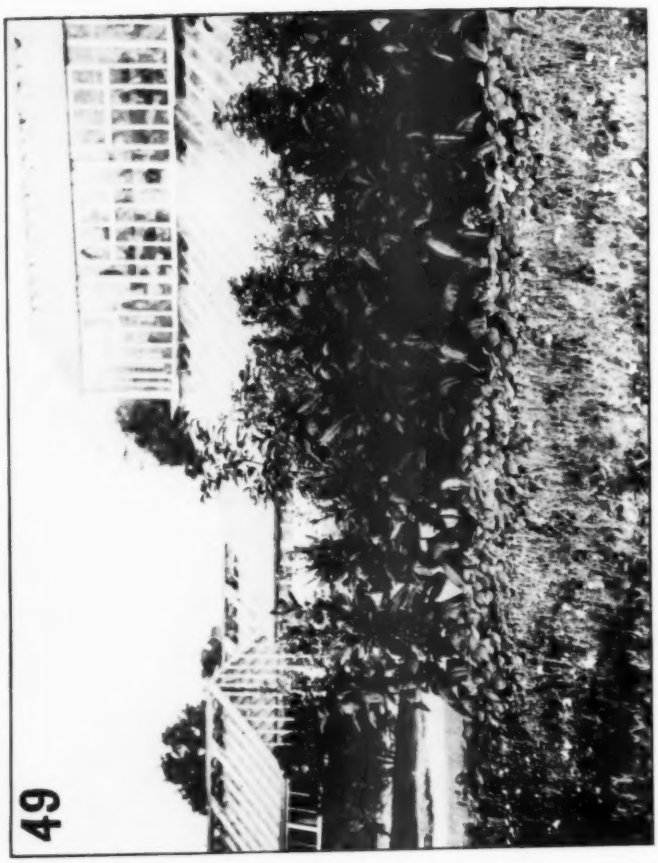
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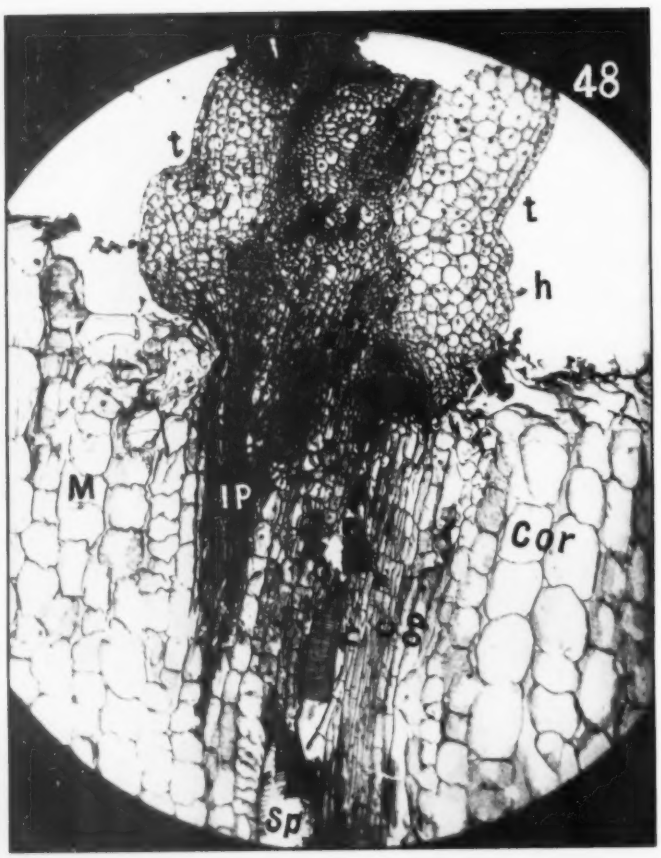
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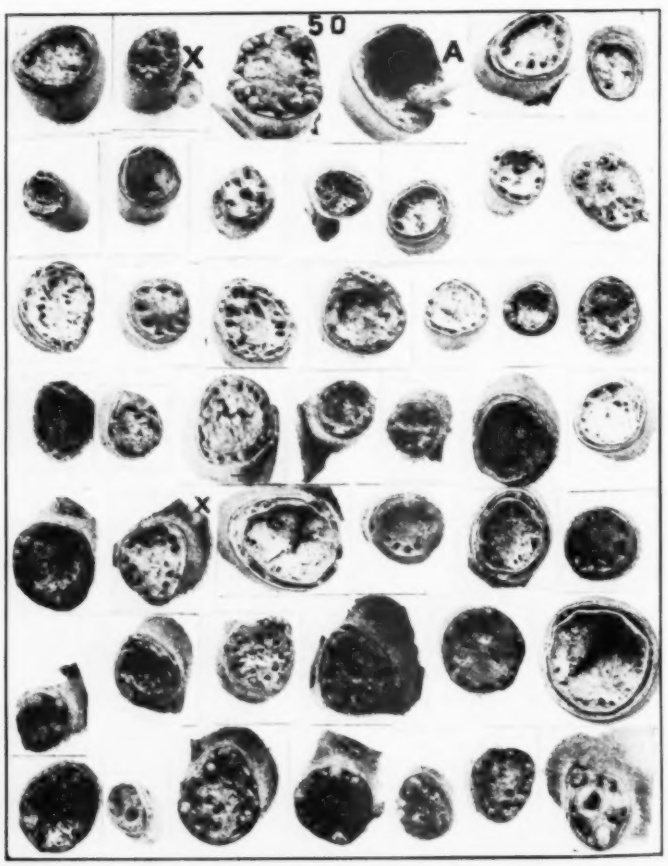
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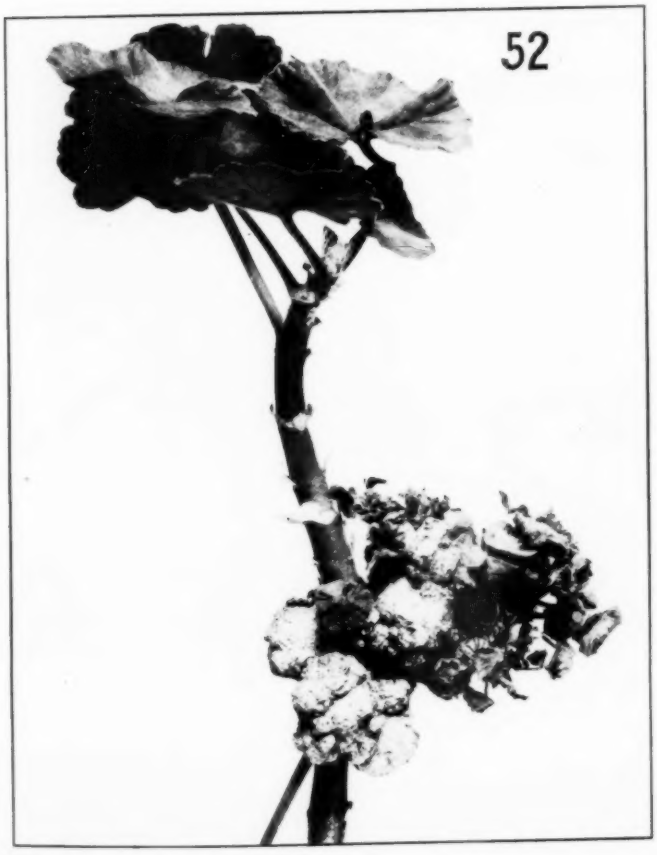
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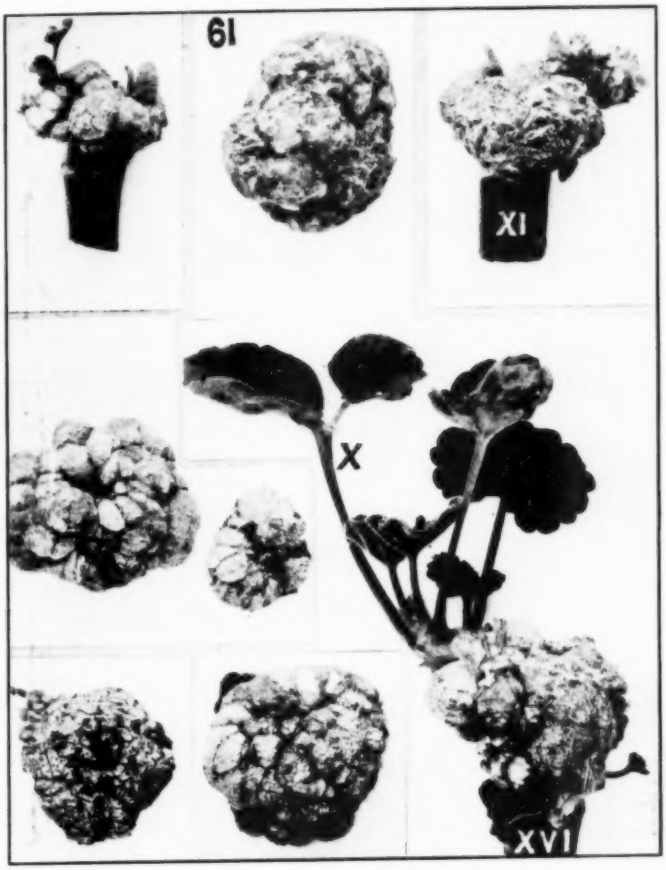
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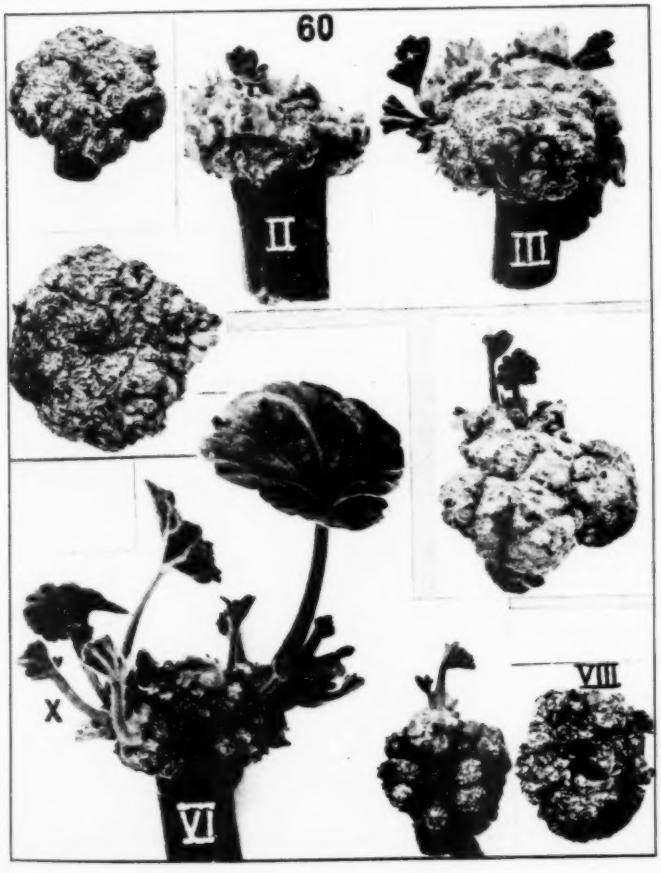
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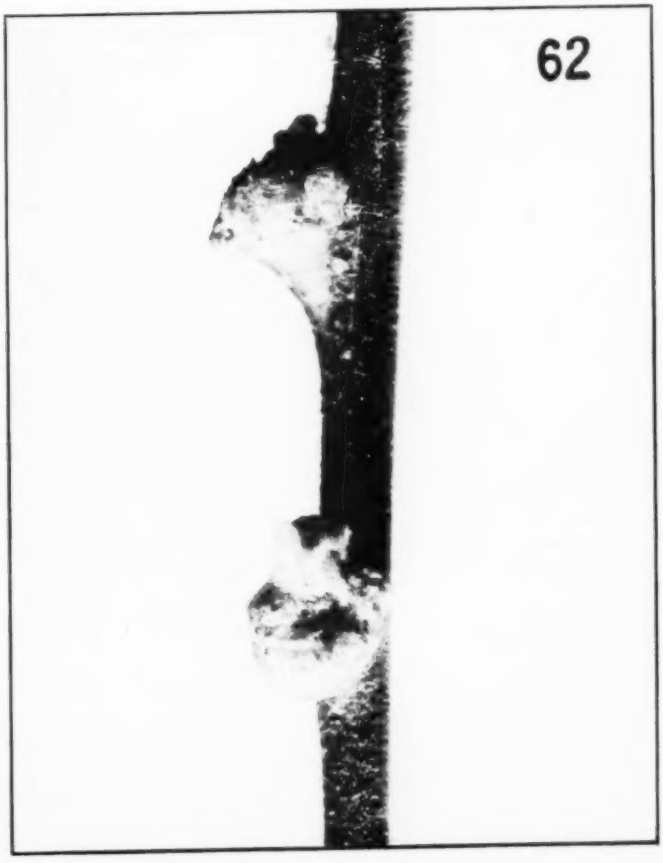
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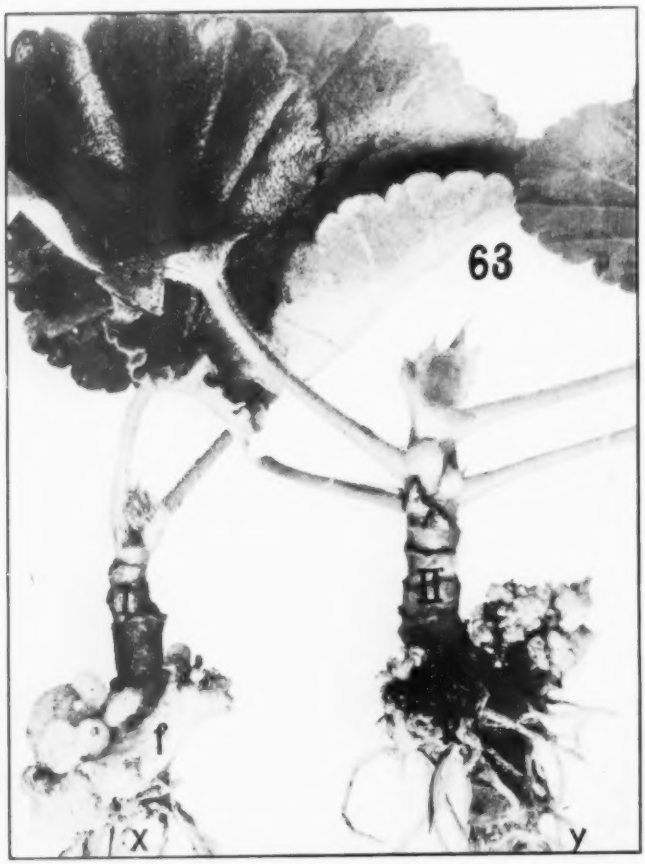
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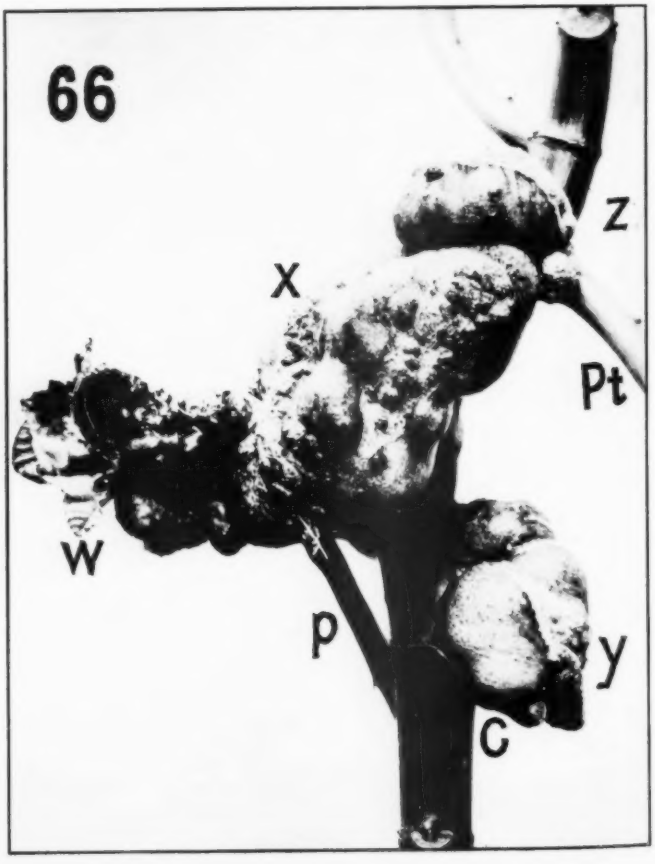
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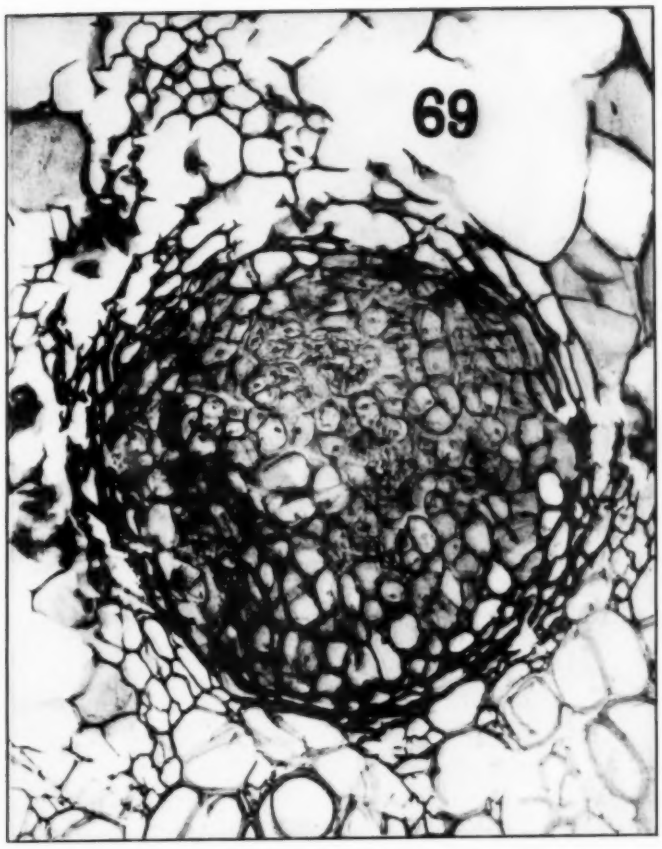
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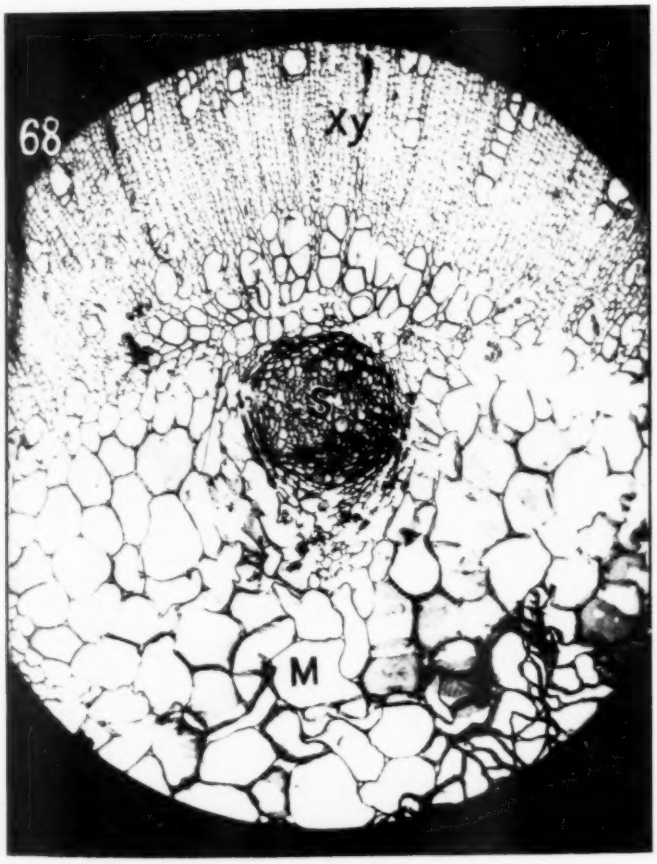
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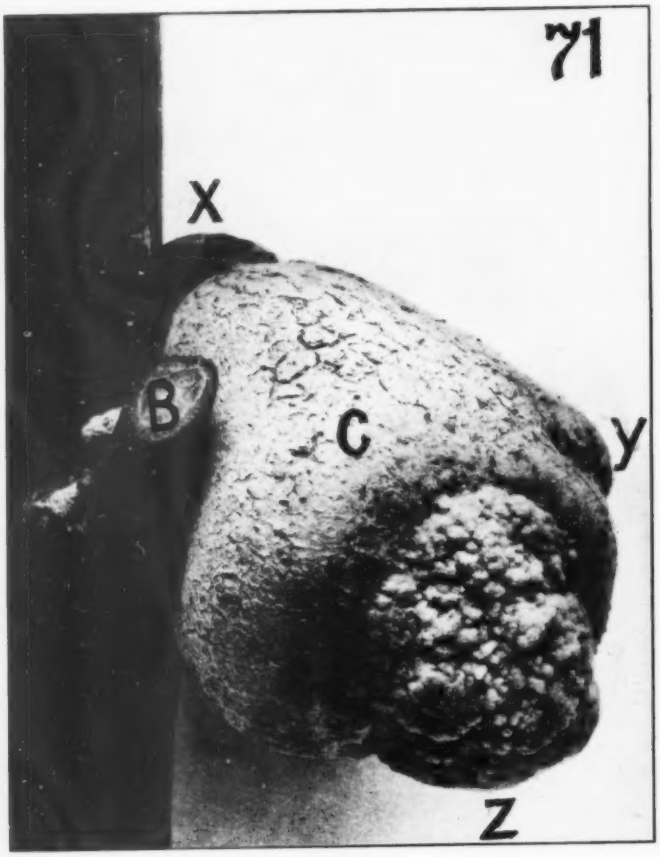
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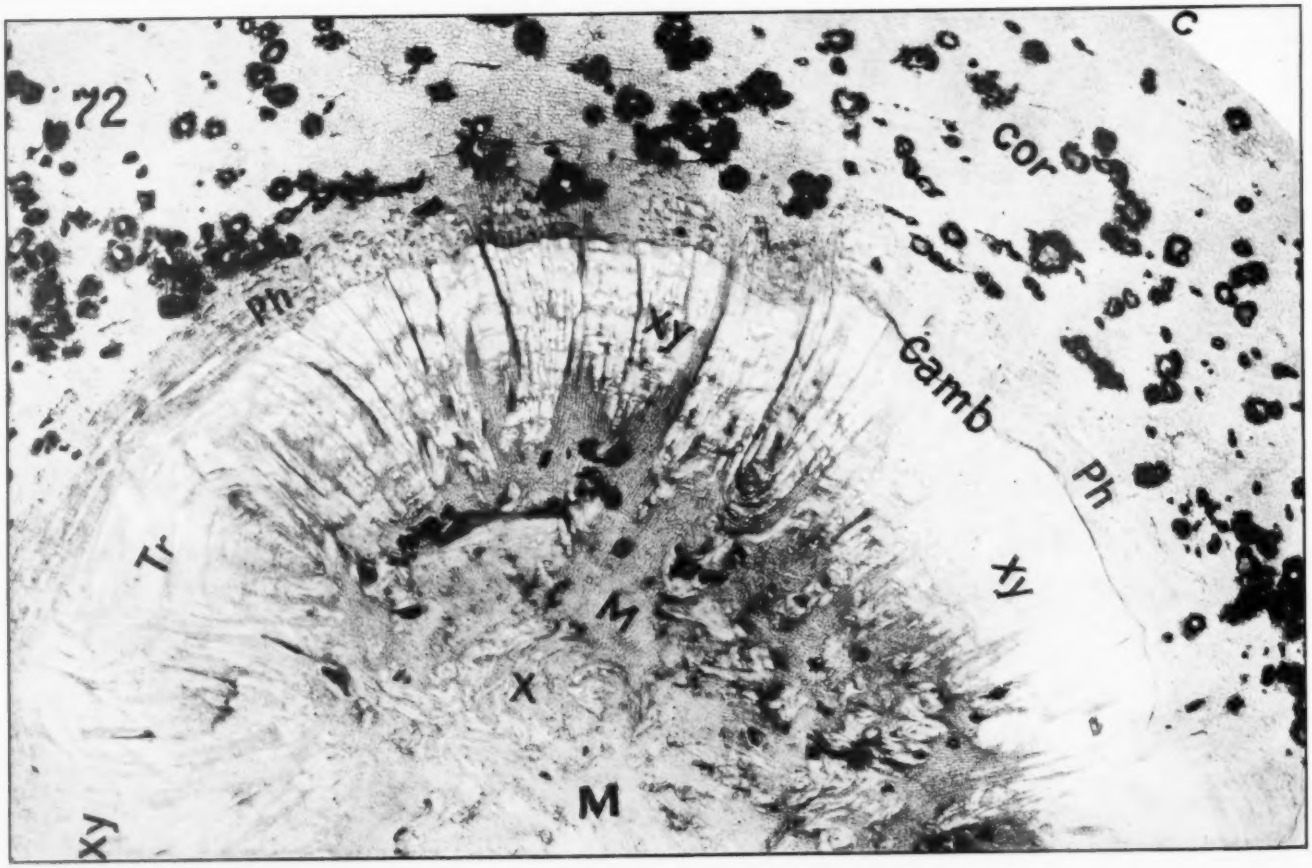
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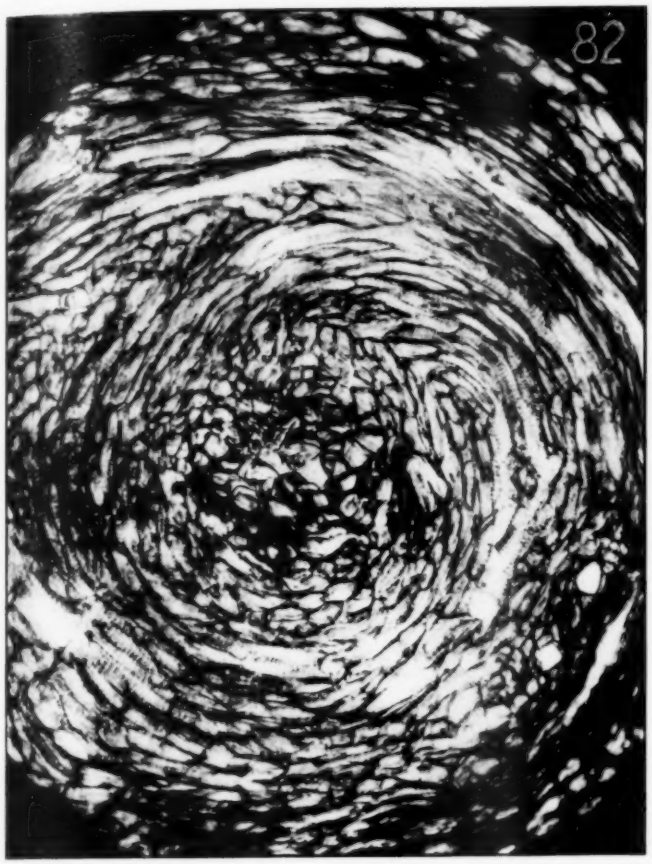
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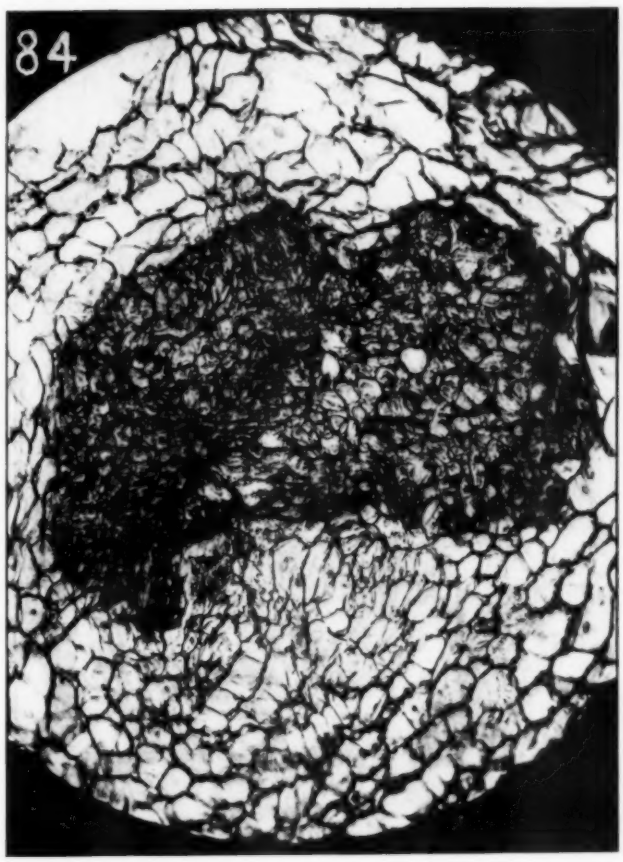
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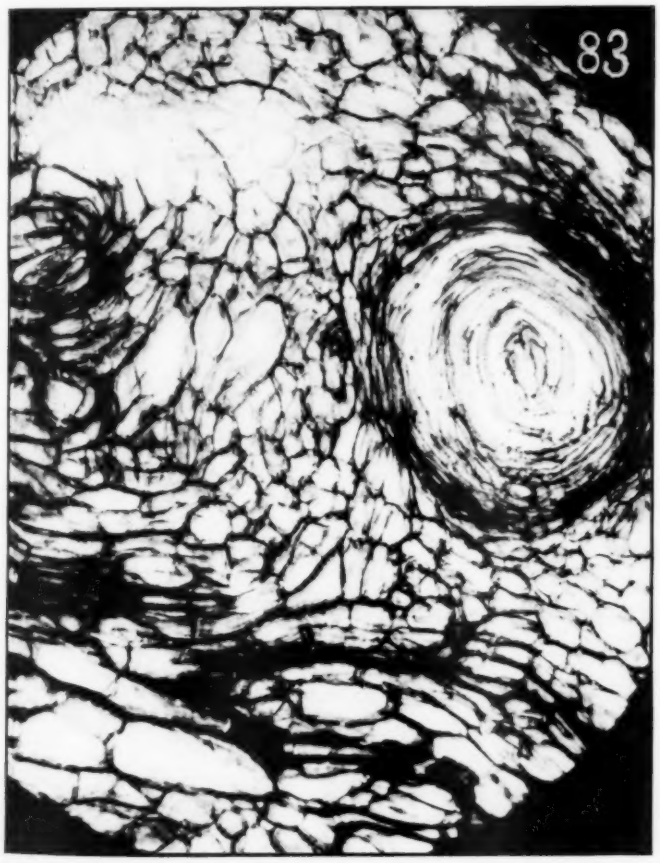
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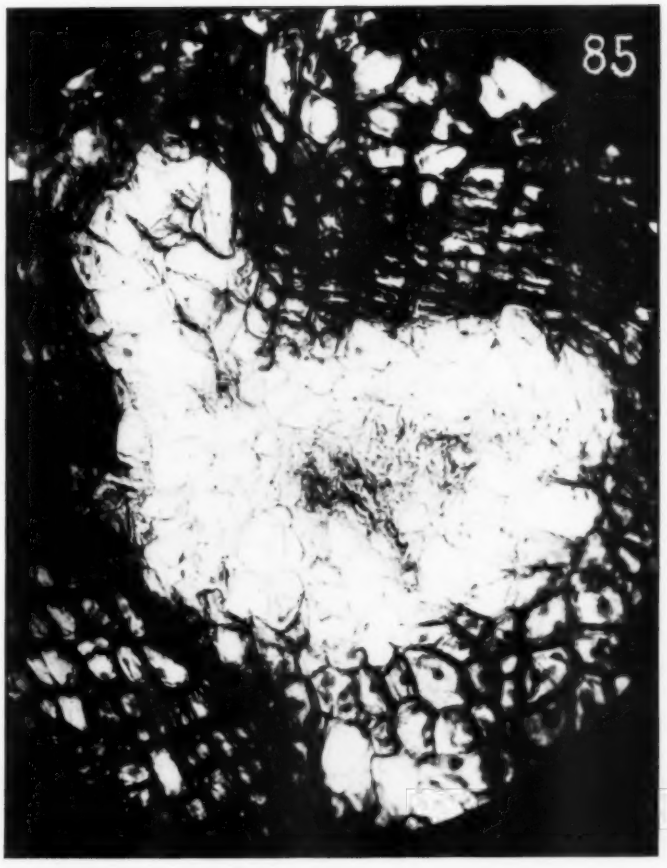
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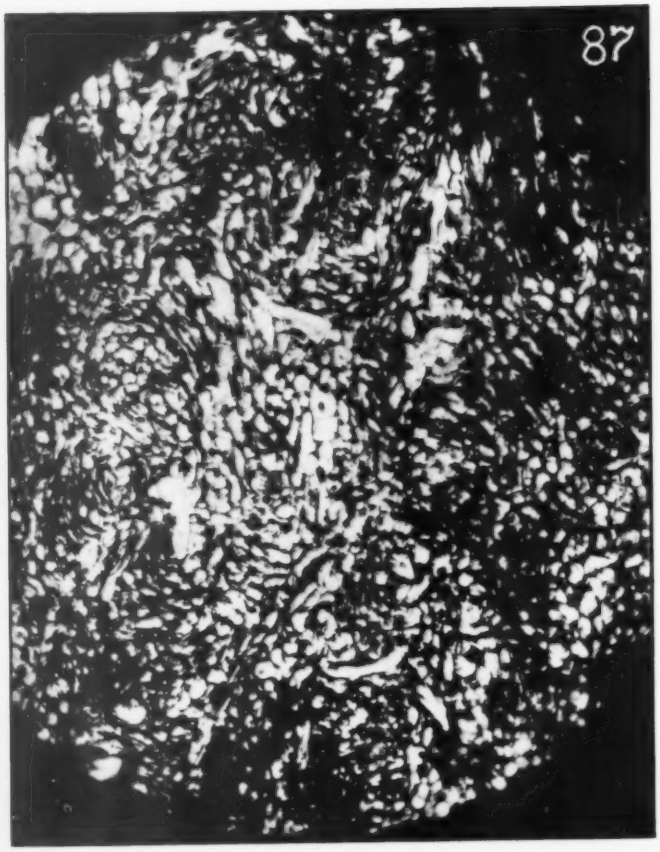
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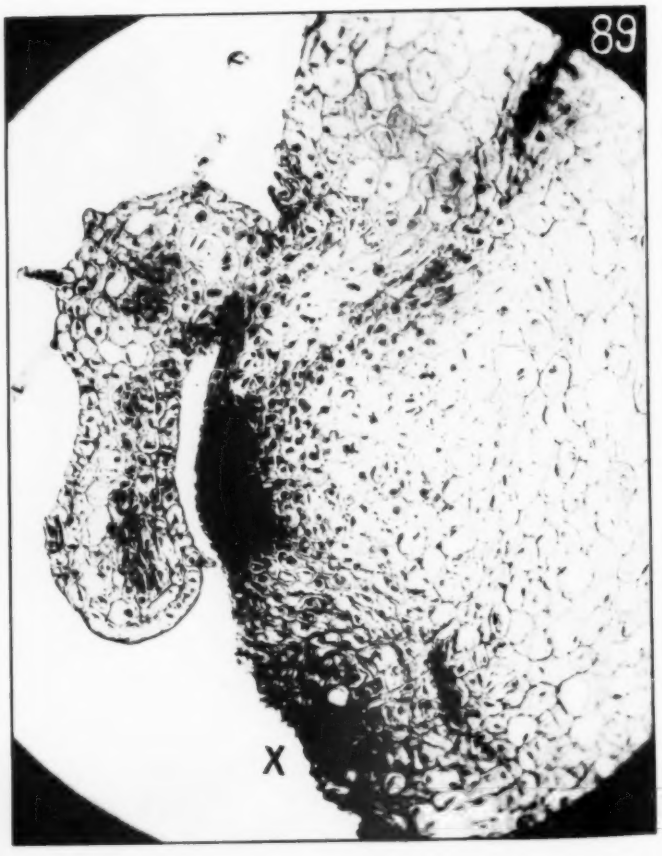
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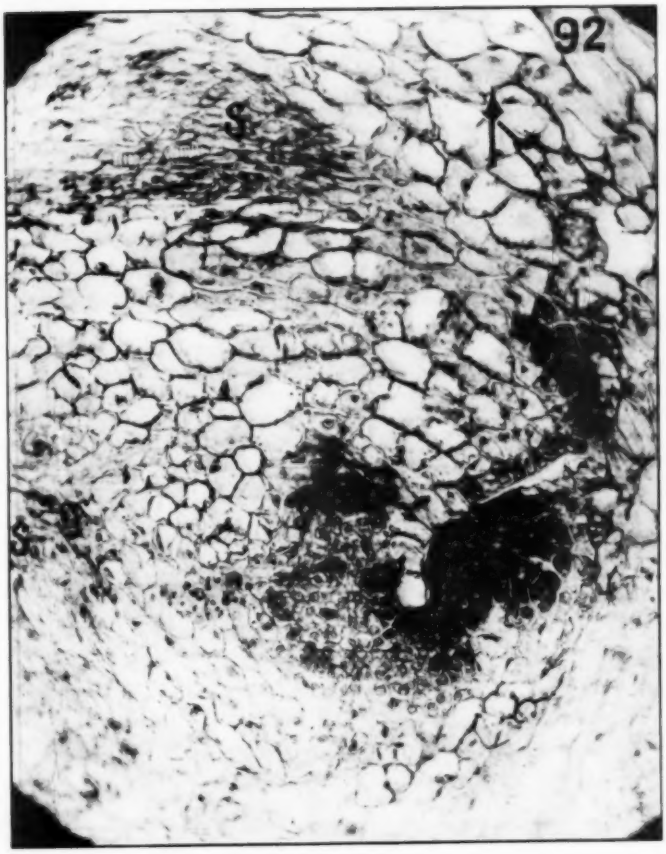
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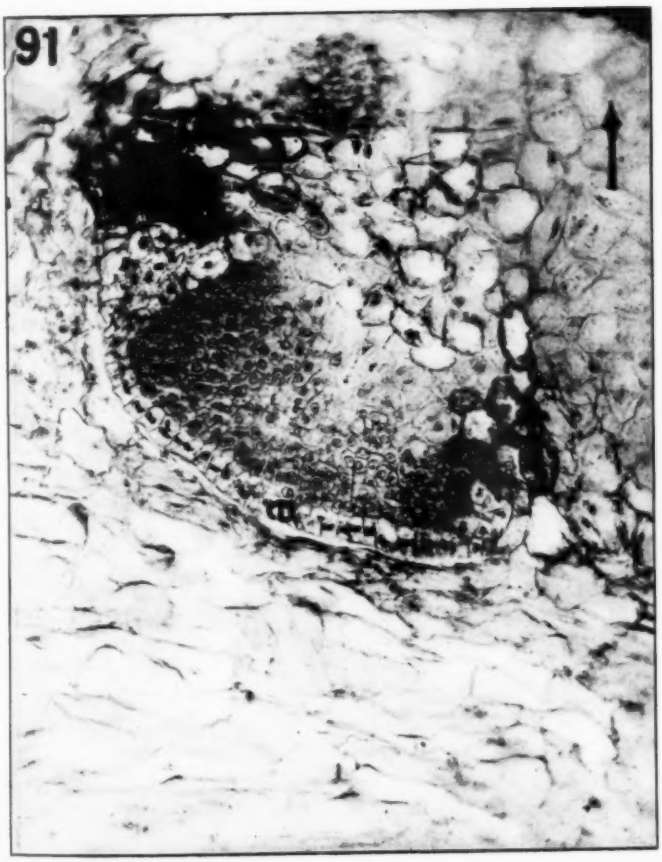
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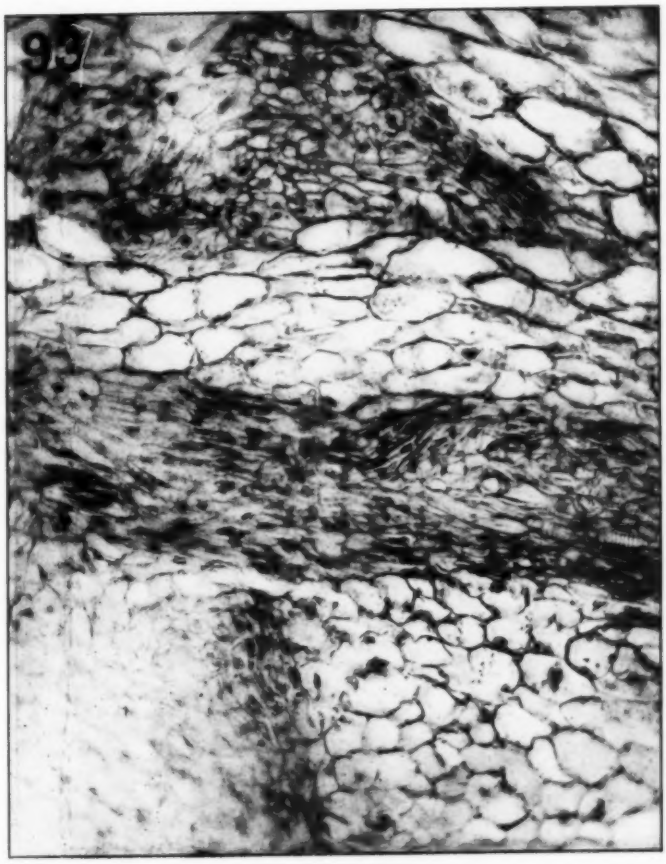
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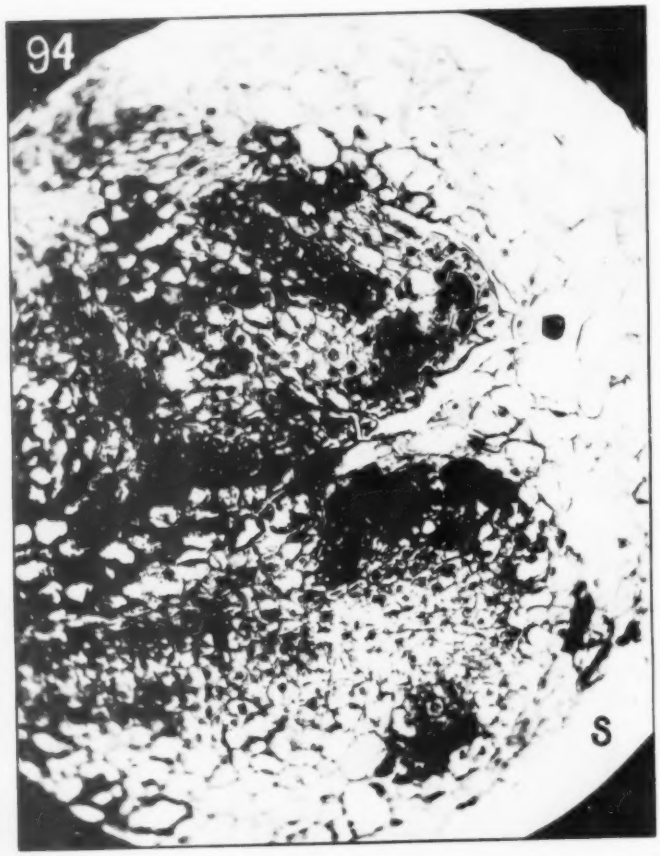
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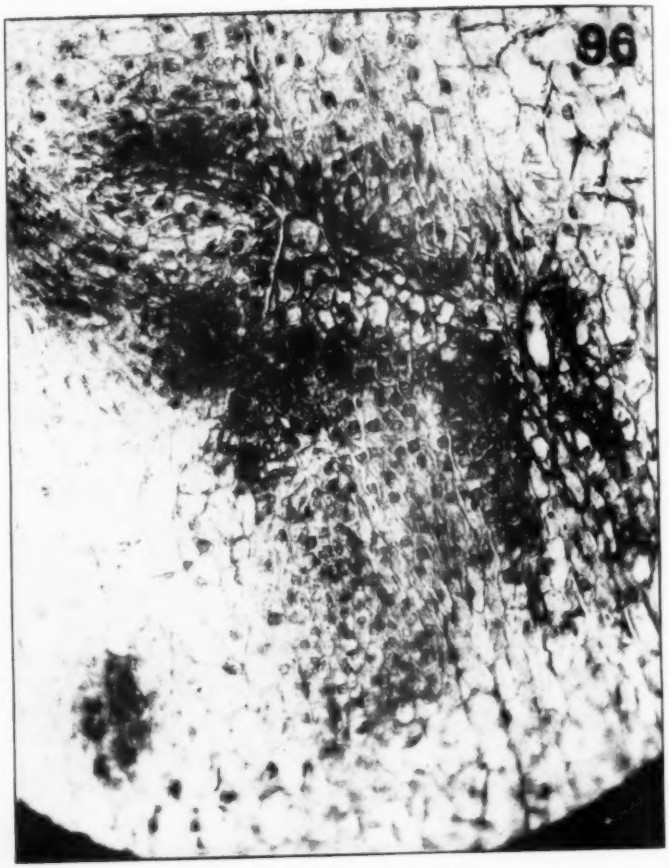
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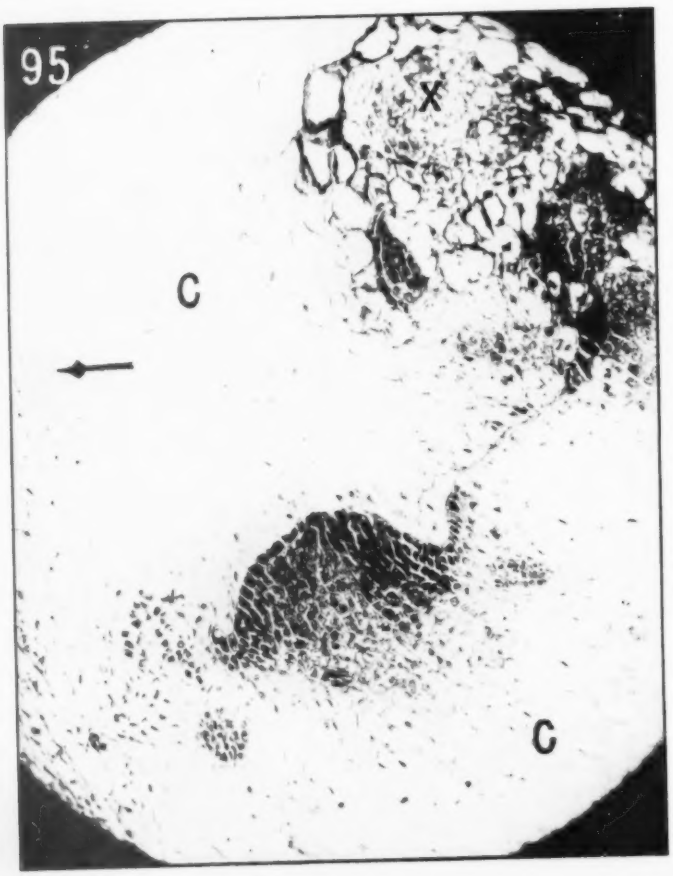
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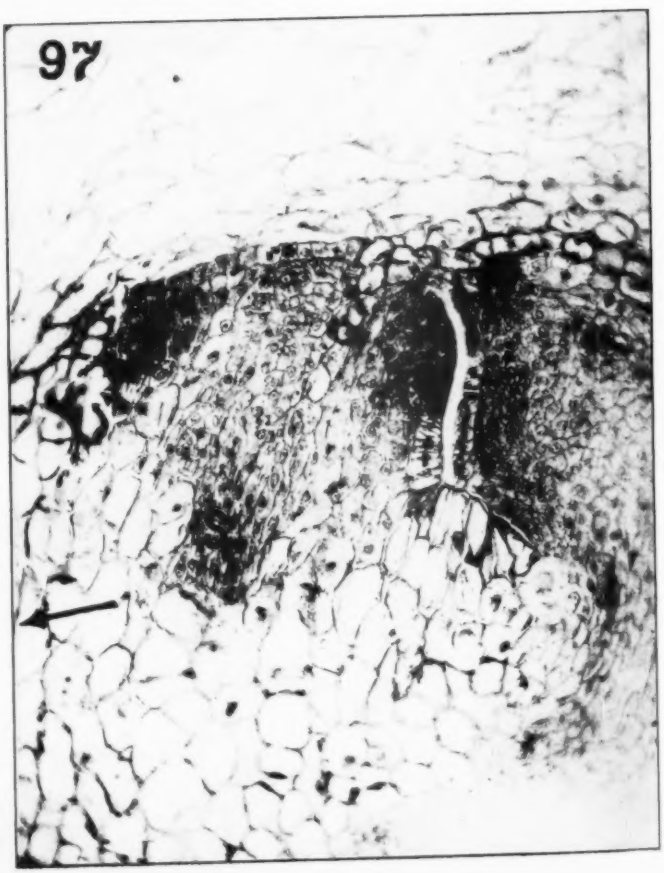
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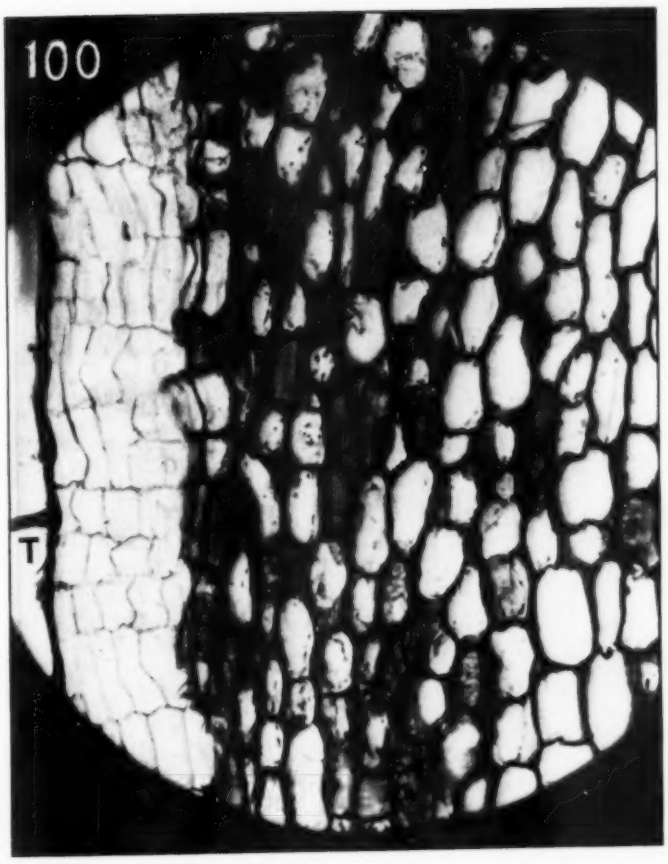
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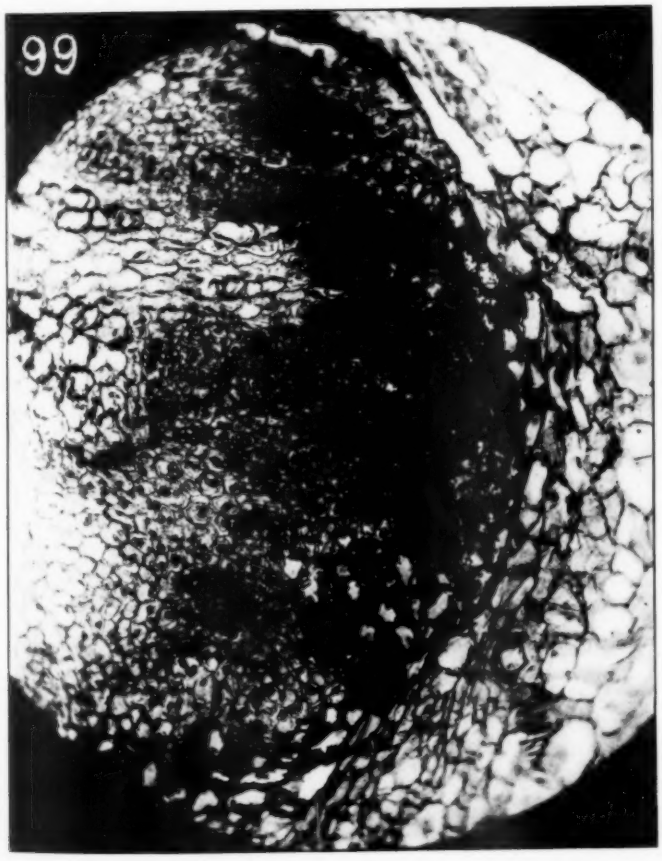
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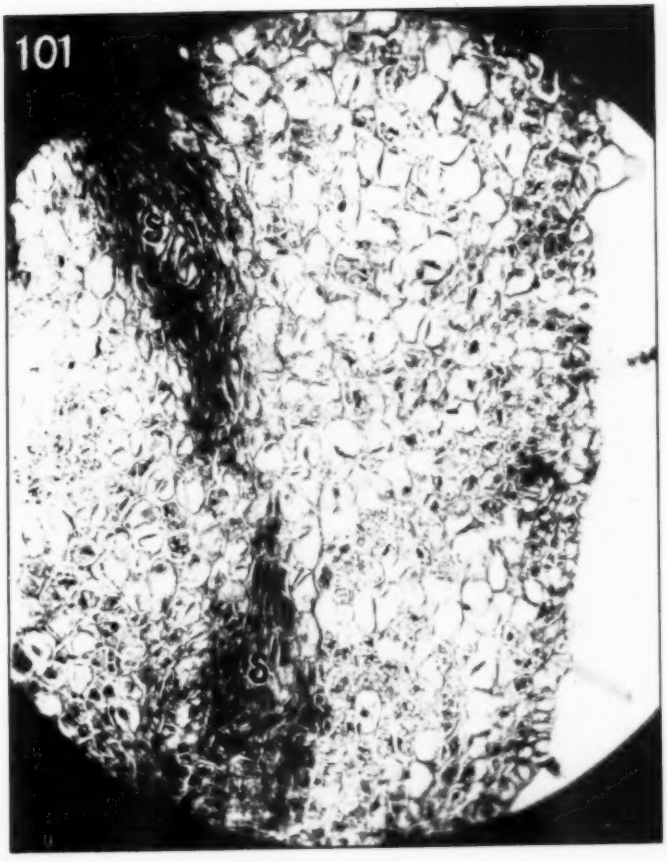
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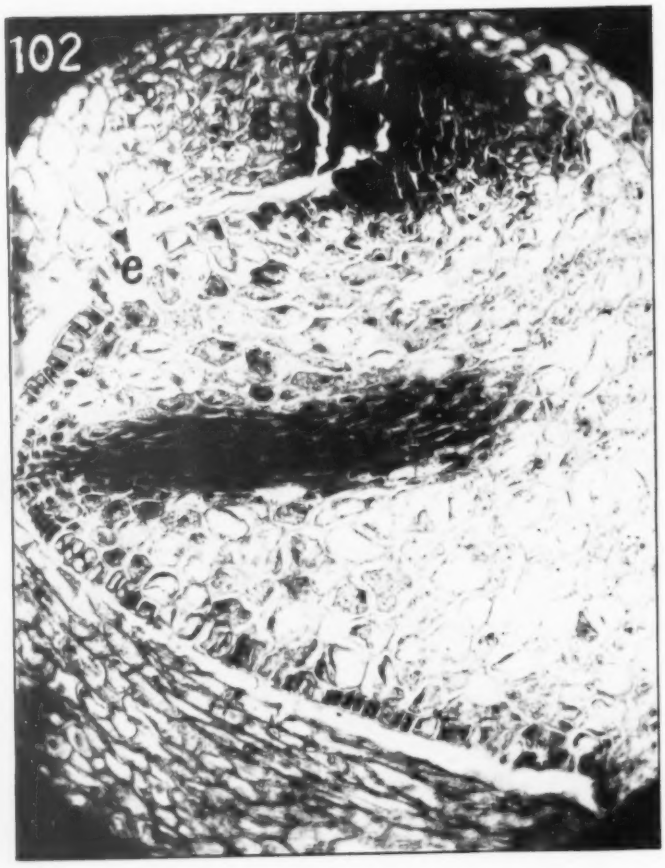
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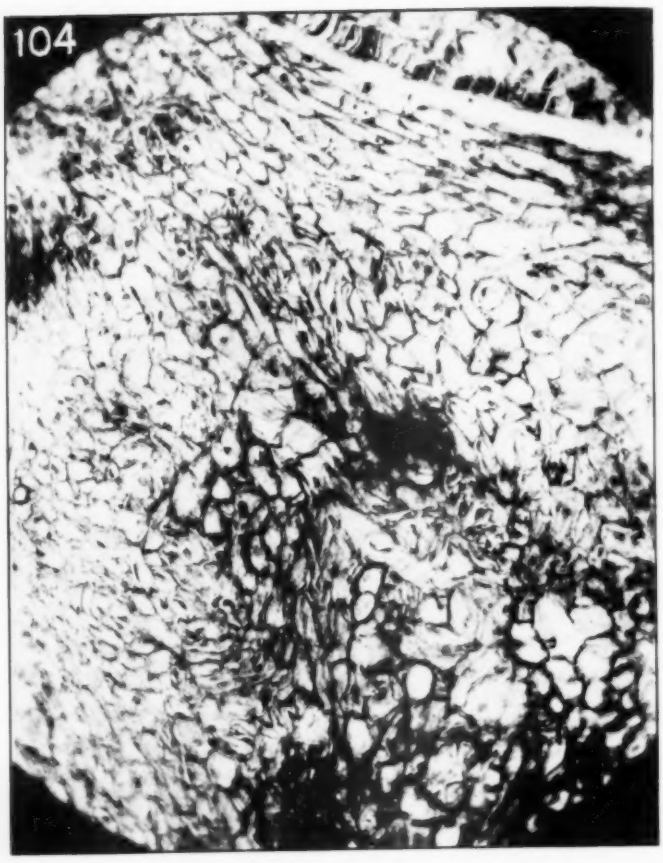
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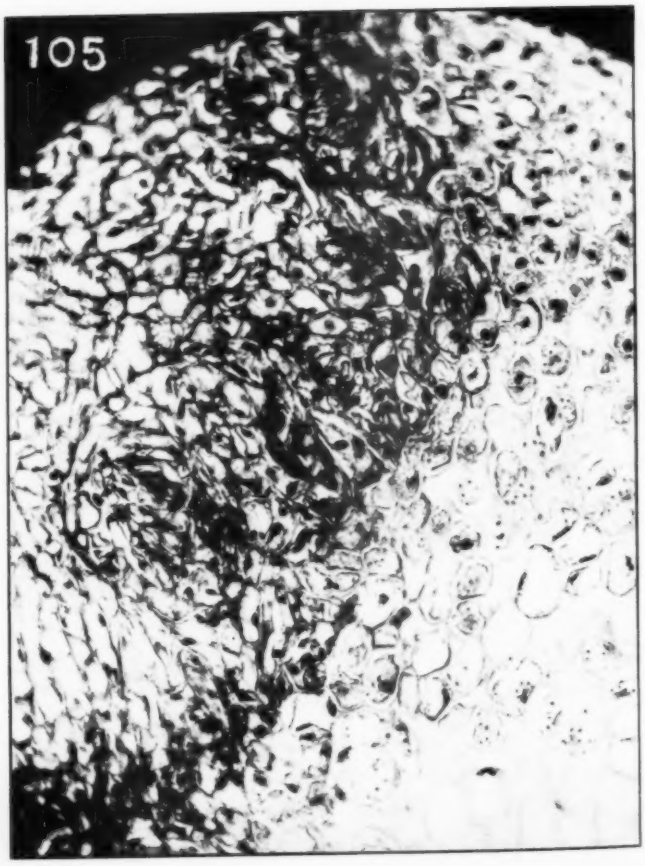
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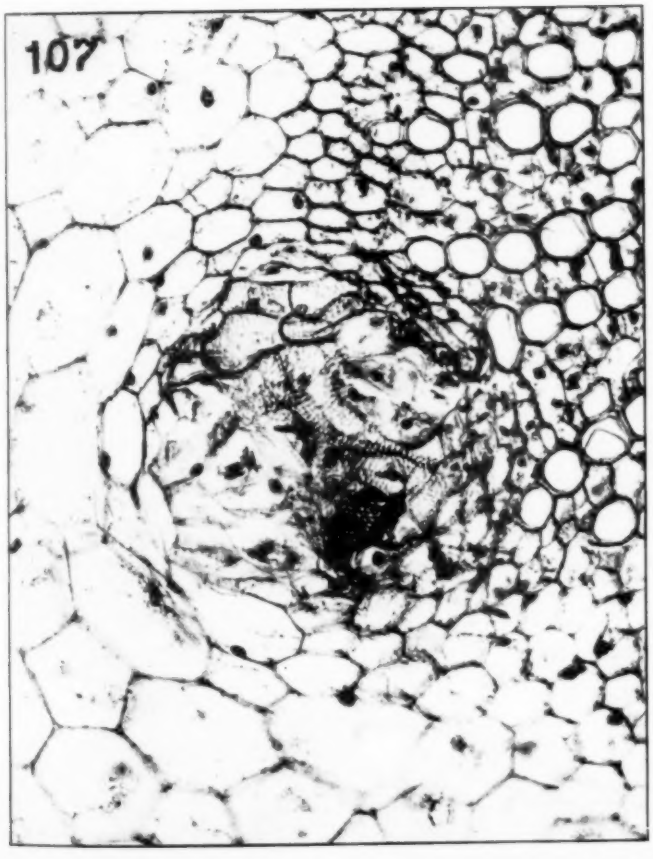
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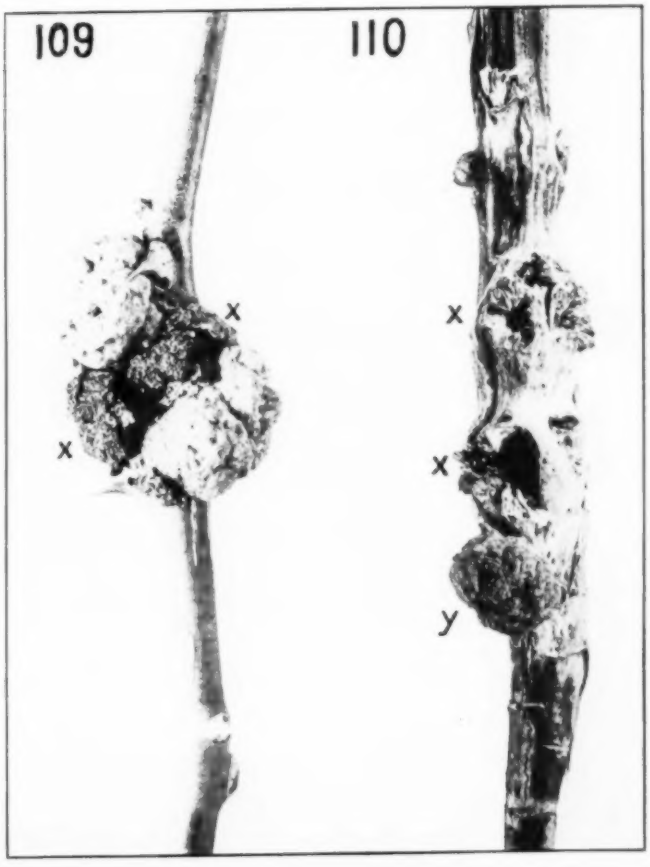
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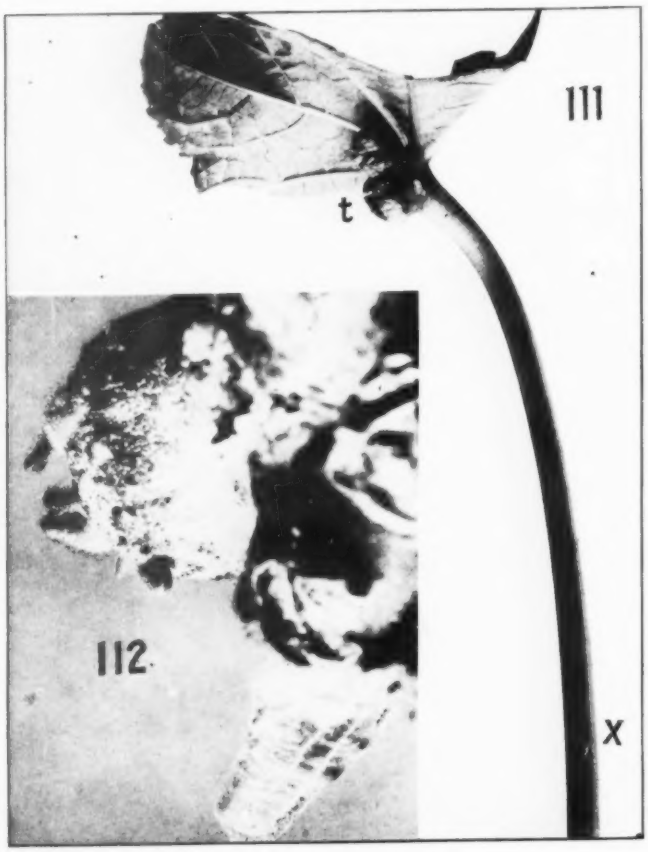
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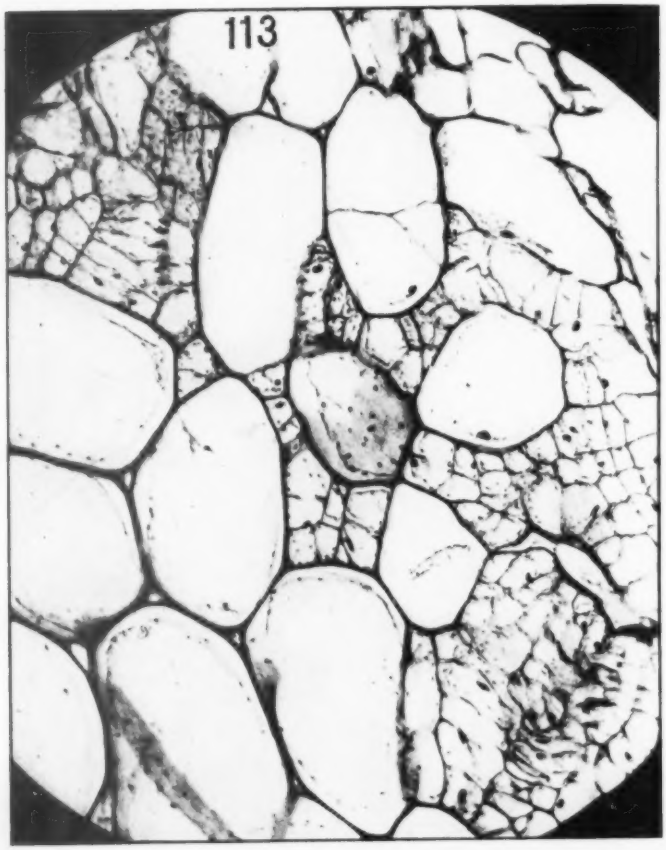
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FIG. 78.—Leaf-axil crown-gall teratoids on cauliflower. Inoculated by needle-pricks, September 29, 1916, with the hop strain of *Bact. tumefaciens* (Colony 1 from sunflower inoculation of 1915). Front view. Left tumor commencing to decay. The tumors bear both roots and leafy shoots, the middle shoots being knobby (internally sarcomatous) while the upper shoot on the left-hand tumor is wholly sarcomatous. Photo. January 2, 1917. (× 2 *circa.*)

FIG. 79.—Back of Fig. 78. Time 95 days. The shoots at the left which looked normal in Fig. 78 are here seen to be tumefied at their base, and the lower one also in its middle part. (× 2 *circa.*)

FIG. 80.—Pelargonium internodal crown-gall teratoid. Planar enlargement of a section through II of Fig. 60, in the part containing both diminutive leafy shoots and red (floral) pigment. Stem inoculated August 1, 1916, cut and fixed September 6. Slide 1282-19. Cortex and cambium both involved, wood much thickened. The arrows indicate approximately the surface of the cut stem. Cortex on left side, pith on right. Bulk of the tumor sarcomatous. Twisted (somewhat torn) teratoid elements at the top. For details consult Figs. 81 to 87. Figure 86 is from the white area in the center (¾ inch from the top of the plate) and the large grayish, fine-celled mass below it is sarcoma.

FIG. 81.—Pelargonium crown-gall teratoid. Thickened and distorted (criss-crossed) cambium from base of the tumor shown in Fig. 80. Tracheæ on right, sieve tubes on left. Slide 1282-6. (× 160.)

FIG. 82.—Woody whorl in Pelargonium tumor. Tracheæ numerous; sieve tubes on periphery. Slide 1282-6. (× 160.)

FIG. 83.—Two smaller whorls in cortex of same tumor as in Fig. 82, and one field of the microscope above the latter. The left one is quite immature. Slide 1282-6. (× 160.)

FIG. 84.—Mass of deep-staining, sarcomatous tissue lying in modified cortex and connected with the main mass by a pedicle of coarser cells. Slide 1282-10. (× 150 *circa.*)

FIG. 85.—Central degeneration in Pelargonium teratoid tumor. The white tissue was dead and shriveled in its center when it was fixed. Stained with methyl green and acid fuchsin. Slide 1282-6. (× 150 *circa.*) Dead tissues do not take the fuchsin stain.

FIG. 86.—Coarse-celled, large-nucleated living tissue (cortex ?) near surface of Pelargonium tumor shown in Fig. 80, surrounded by the rapidly proliferating small cells of the sarcoma. Slide 1282-18. (× 150 *circa.*)

FIG. 87.—Middle of tumor in Fig. 80 showing rapidly proliferating small-celled, sarcomatous tissue which contains a few distorted vessels (tracheæ). Slide 1282-24. (× 150 *circa.*)

FIG. 88.—Planar enlargement of vertical section of axillary Pelargonium teratoid inoculated October 25, 1915, and fixed February 28, 1916. Block 1187 (second piece), slide 12. The photograph is variously lettered to indicate approximately the location of the following photographs showing teratoid fragments growing from the surface of the tumor (*W*) and buried in its thickened cortex, which extends as far inward as the middle curved line of xylem (*xy*), beyond which is modified pith (*m*) containing many distorted tracheæ (wood vessels).

FIG. 89.—Detail from Fig. 88 at *W* showing an abortive surface organ. The tumor is naked, sarcomatous and disintegrating at *X*. At the right are normally oriented cortex cells. Slide 1187-12. (× 160.) The dark part under the sprout is embryonic tissue.

FIG. 90.—Buried embryonic tissue covered by a membrane and lying upside down unconformably on other tissues. The arrow points toward the surface; sarcoma cells at *S*. Lining membrane at *e*. In the upper (dark) part, which represents embryonic tissue, epidermis faces epidermis. From Fig. 88 at *A*. (× 150 *circa.*)

FIG. 91.—Small fragment of embryonic tissue showing its membrane (epidermis) at *e* lying unconformably on another tissue. The arrow points toward the surface of the tumor. Block 1187. From Fig. 88 at *B*. (× 200 *circa.*)

FIG. 92.—Another small fragment of embryonic tissue, capped by a trichome, and buried unconformably in a second mass of younger embryonic tissue. The surface lies in the direction of the arrow. The best developed part is covered by a membrane; the deep-staining younger part is not covered. The sarcomatous tissue is at *s. s.* From Fig. 88 at *V* but from slide 1187-19. (× 175 *circa.*)

FIG. 93.—Extreme upper left part of Fig. 92 for the sarcomatous portion, and also half a field nearer the surface to show another finger-like portion of the sarcoma lying in the cortex. Slide 1187-19. (× 200.) For orientation in connection with Fig. 92. The finger-like central mass of disorderly cells ends abruptly (like the upper mass) in a coarse-celled cortex ½ inch beyond the right margin of the picture. See upper *S* of Fig. 92.

FIG. 94.—From Fig. 88 at *Z*. Lobes of embryonic tissue in cortex. These point toward the surface (*s*). Their top is covered by a membrane and one of them bears a glandular hair. Slide 1187-12. (× 160.) The dark color is due to the deep red (fuchsin) stain.

FIG. 95.—From Fig. 88 at *Y*, showing membrane-bearing embryonic fragments lying in cortex; the dark parts are embryonic tissue; *s*, sarcoma; *c, c*, cortex. Surface lies in direction of arrow. Slide 1187-12. (× 160.)

FIG. 96.—From Fig. 88 at *R*, which is two fields of the microscope deeper in the tumor than Fig. 92. It shows small fragments of embryonic tissue (tooth-shaped pieces) bounded by a membrane pointing away from the surface, lying unconformably on other embryonic cells and surrounded by coarse-celled cortex. In the upper dark part at the extreme left vessels are visible. This part ends abruptly in a coarse-celled cortex just beyond the part here shown. Slide 1187-19. (× 200.)

FIG. 97.—From Fig. 88 at *S*, but on slide 1187-14. Three small tooth-shaped pieces of embryonic tissue covered by epidermis and lying unconformably in cortex. Tissue sarcomatous at *S*. The surface lies in the direction of the arrow. (× 200.)

FIG. 98.—Embryo fragments in cortex near surface of tumor. Sarcoma at *S* but mostly out of focus. Block 1187. Slide misplaced, probably near No. 14 or No. 15. Possibly 17. (× 160.)

FIG. 99.—From Fig. 88 at *T*. Slide 1187-12. Deep-staining embryo fragments lying unconformably in cortex. (× 150 *circa.*)

FIG. 100.—Longitudinal radial section through outer normal part of a Pelargonium stem (below a tumor) for comparison with Figs. 82, 84, 86, and enlarged (tumefied) cortex shown in Fig. 101. This shows surface (cork layer) at the left and then the ordinary cortex cells of the young stem. Trichome at *T*. Slide 1282-24. (× 150 *circa.*)

FIG. 101.—Outer part of overgrown cortex of a Pelargonium teratoid tumor. Plant inoculated October 19, 1915, and material fixed January 11, 1916. Surface of tumor at right covered by an irregular epidermis bearing hairs. The cells are smaller than normal cortex cells (Fig. 100) and many are filled with starch, but are normally oriented or nearly so. The excess of starch means that more sugar passed into the tumor than could be used for growth, the surplus in such cases being converted into starch and stored as a harmless product. Sarcomatous elements occur at *S, S*. Slide 1166-15. (× 150 *circa.*)

FIG. 102.—Slide 1166-15 immediately under (at left of) Fig. 101. It shows a small tooth-shaped piece of tissue (a bud) pointing exactly away from the surface of the tumor. This has a well developed epidermis (*e*) and incipient vessels (*V*). Sarcoma cells occur at *S*. The tissues above and below this bud are shown in Figs. 103 and 104.

FIG. 103.—Photomicrograph from slide 1166-15 immediately above Fig. 102, showing disorderly (sarcomatous) arrangement of the cells. At the top on the right are cortex cells (*C*). The dark cells in the extreme lower part (*e*) are deep-staining normally oriented embryonic cells, *i. e.*, the same as those shown in the upper part of Fig. 102. The rest of the tissue is sarcomatous. (× 180 *circa.*)

FIG. 104.—Slide 1166-15 immediately below Fig. 102, a small portion of which is included at the top. This field also is largely sarcomatous. (× 160.)

FIG. 105.—Same tumor as in Fig. 104, but from another part nearer the surface. The section shows on the left the deep-staining, imperfectly vascularized, disorderly cells of the sarcoma, and on the right the non-sarcomatous but overgrown cortex. Slide 1166-22. (× 180 *circa*.)

FIG. 106.—One lobe (enlarged) of that part of the nasturtium tumor shown at *Z* in Fig. 76. Numerous roots can be seen pushing out of the otherwise naked body of the tumor. A little of the stem is visible at the extreme left. Inoculated August 5, 1916. Photo. November 13, 1916. (× 6.)

FIG. 107.—Paris daisy stem between tumors, in cross-section, showing outer part of pith (at left) and inner edge of vascular bundle. In the center of the figure is a tumor strand here consisting mostly of disoriented pitted vessels (tracheæ) to the right of which are the normal spiral vessels and medullary rays of the inner face of the bundle. There are normally no tracheæ in this part of the bundle. The black dots are nuclei. Slide 639-A-19. (× 210.)

FIG. 108.—Inoculated crown gall on rose. Not a teratoid. The organism used was isolated from tumor on a hot-house rose received from Massachusetts. Inoculated June 2, 1916. Photo. September 21, 1916. (Natural size.)

FIG. 109.—Massachusetts gall on rose. The primary tumor, *XX*, was produced by a bacterial inoculation made June 16, 1916. This tumor was treated January 10, 1917, with a caustic oil and destroyed, but new tumors have arisen from its base. Photo. May 16, 1917. (Natural size.)

FIG. 110.—Paris daisy stem killed by crown gall. It was inoculated April 7, 1911, at *X*. *X*. from a colony plated from a secondary gall. Tumors developed at the sites of inoculation and later sloughed off. Subsequently a new tumor appeared at *Y*. This tumor, together with the whole stem, was dead when the photograph was made, January 23, 1912. Time 10 mos., nearly.

FIG. 111.—Ricinus leaf inoculated on the blade with the hop strain of the crown-gall organism. Tumors at *t* on both upper and under surface of the blade. *X* is the level of the cross-section shown in Figs. 68, 69. (Natural size.)

FIG. 112.—Pelargonium stem teratoid showing glandular, hairy, abortive red outgrowths (floral anomalies) protruding from the surface of the tumor. This is a side view of Plate XV, Fig. 57, in *The Journal of Cancer Research*, April, 1916.

FIGS. 113, 114.—Figures showing imperfect development of one side of a secondary tumor in the petiole of an inoculated Paris daisy. The larger cells are the normal cells of the cortical parenchyma of the leaf stalk. Surface of petiole at right in 113 and at left in 114. Slide 590-B-13. (× 160.)

FIG. 115.—Margin of crown-gall tumor from inoculated disk of the sunflower. The tumor is an extremely vascular growth developed from the torus (thin vascular layer which bears the seeds), but lying in the pith. The whole is an invasion. P, P, designate pith cells and the arrows the marginal advancing portions of the tumor stroma and tumor cells. Some of the vessels are so young as still to lack woody deposits and still to contain nuclei, as the one designated by the crossed arrow. Slide 1147-C-6. Stained with acid fuchsin and methyl green—sarcoma cells red, vessels blue, pith cells white. (× 160.) May be compared with Figs. 6 and 52 of my paper in *The Journal of Cancer Research* (April, 1916), which were made from the same series of sections.

# THE CARREL TREATMENT OF WOUNDS APPLIED TO CIVIL PRACTICE.

By Jos. S. Lawrence, A. M., M. D.,

*With the American Ambulance, Neuilly, France, Summer, 1916.*

The Carrel-Dakin method of treating infected wounds depends for its success so largely upon the technique with which it is applied, that it seemed advisable to write out carefully the various steps, paying particular attention to the bacteriological phase. The surgical phase has been treated exhaustively in a number of recent articles, but the bacteriological part has usually been stated very briefly. It is very necessary that careful attention be given to the bacterial content, which should be regularly ascertained, as a guide for the surgeon. Secondary infection may readily occur after the wound has been under treatment even in the hands of the most skillful attendants, and for its detection the bacteriological charts are found more serviceable than the temperature records. The method here described is simplified so that it may be used for the treatment of one case or of many cases, for its employment in hospitals or for use in the dispensary or office on ambulant patients. It is the technique which is employed in The Johns Hopkins Hospital.

In order to secure satisfactory results from the employment of the Carrel method, it is absolutely necessary that the following points be given the strictest attention. First, Dakin's solution, the fluid used for irrigation, must be very carefully prepared according to the formula described by Daufresne.[1] It breaks down readily when exposed to the light or air and hence should be kept well corked in colored glass bottles, never in metal containers. A preparation more than a week old should not be used. This solution is not a powerful disinfectant but a mild antiseptic. Second, this is not a system of drainage, but of irrigation or laking. Third, a knowledge of the efficiency of the treatment can be obtained only by systematic, accurate and careful bacteriological observations.

The method can be employed with success in the treatment of most flesh wounds, abscesses, compound fractures or chronic osteomyelitis. We feel strongly that it can be used and is indicated in most cases where at the present time drainage is instituted. It may be considered prophylactic or therapeutic according to the nature of the case. If the wound has been freshly made in sound tissue, the object will be to prevent the development of infection; but if the case is one of chronic abscess or osteomyelitis, the aim is to eradicate the infection. The steps in the method are about as follows: If the wound is the result of an accident and is filled with dirt,

[1] J. A. M. A., 1916, Dec. 9, LXVII, 1777.

shreds of clothing and other foreign bodies, all of the débris should be removed with forceps and by scrubbing with green soap and sterile water. A brush can be used for this purpose if necessary, and shreds of tissue that are without a blood supply should be excised. If we have a puncture or penetrating wound that is more extensive beneath the skin than on the surface, it should be opened so that thorough irrigation may be established. The object here is to prevent the multiplication of any bacteria in any part of the wound among the many that were carried in by the accident. In the presence of Dakin's solution, when properly made, no bacteria can flourish, their development is inhibited, while the body tissues are not hindered in their repair work. Phagocytosis, as a rule, is observed to take charge of the introduced organisms. Healing of the wound by first intention can be expected in many cases. If, however, the wound is more than six hours old, or we have a chronic sinus discharging pus, the problem is a little more difficult. The wound should be thoroughly cleansed, foreign bodies removed, necrotic tissue cut away and the surface opening increased, if necessary, in order to secure the best results from the irrigation. A counter opening should be avoided when possible in deep wounds, and in penetrating wounds the perforation farther from the main injury should be encouraged to close early. The object is always to secure thorough flooding of the injured tissues at each irrigation. To help to insure this, a certain type of rubber tubing, with a caliber of about 4 mm., is used for insertion. It is tied off at one end and perforated at this end with from eight to twelve holes made with a No. 4 leather punch, the first hole being placed as near to the tie as possible and the others arranged spirally and about half an inch apart. Such a tube insures a better spreading of the irrigating solution in a large wound or over a surface wound. If it is a narrow sinus that is to be irrigated, a plain tube open at the distal end and not perforated may be employed. All tubes must be free to move around and must under no condition be packed in with gauze, nor should the surface of the wound be closed in over them. If they are fixed in one position for more than 24 hours the bacteria will multiply rapidly in their bed beyond the irrigating holes. If they are packed in between pads of gauze the bacteria will flourish beneath the gauze, inasmuch as the mechanical effect of the irrigating solution is lost to those parts. The surface must not be closed, for then the free discharge of the surplus fluid and its burden of pus and bacteria will be prevented and any pressure on wounded tissue may produce necrosis, thereby increasing the opportunity for bacterial development. The irrigation should be of sufficient amount to flood the entire wound and a little more, so that the wound may be washed at each irrigation, but care should be taken not to use so much that the outer dressings or the bed are wetted. The irrigations must be made every two hours. A longer interval will permit the dressing to dry in some instances, thus giving an opportunity for bacterial development, whereas a shorter interval will permit too great a flooding of the wound and an irritation of the skin from wet dressings in some patients. The continuous drip was found to be undesirable. Under no conditions should the dressings be removed by any but the attending surgeon, and then only under the strictest antiseptic conditions; usually one dressing a day is sufficient and, when the wound is almost healed, the dressing may be done at two-day intervals if the surgeon is pressed for time. From the beginning of the treatment the wound should be treated as a sterile wound. Only the most accurate aseptic technique will succeed. *Reinfection is always possible either with the same or with different organisms.* This point cannot be emphasized too forcibly, for while the reinfection may not be accompanied with clinical symptoms, yet the implantation of new organisms reproduces the complex conditions of combined infections and

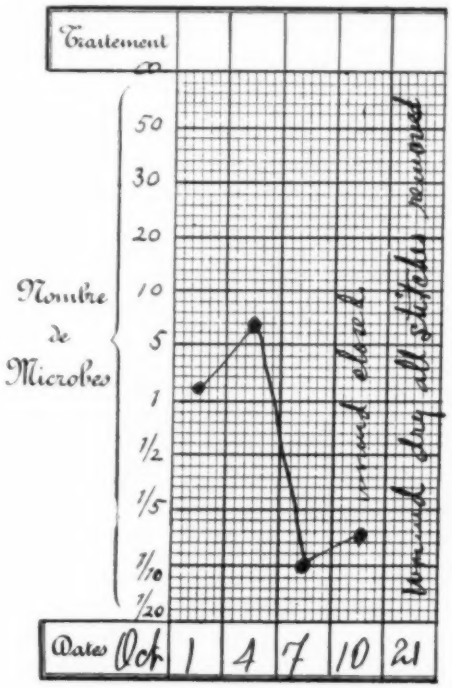


CHART I.

COMPOUND FRACTURE OF THE RIGHT RADIUS.

**The wound was cleaned in thirteen days. For at least four of these days the bacterial count was less than one organism in five fields. On October 10, the edges of the wound were freshened and stitched tight. On October 21, the stitches were removed and the patient discharged to a convalescent hospital with only a simple fracture.**

postpones the day of recovery. The more nearly every wound is treated as though exposed to infection, just as the wound made by the surgeon in operating is exposed to infection, the more satisfactory will the results be. The irrigation can be done by a nurse without disturbing the patient, even while the patient sleeps at night, and this two-hourly interval must be observed throughout the 24 hours. Partly to protect the wound from infection by organisms on the skin and partly to protect the skin from irritation by the Dakin's solution, strips of gauze about four inches wide, previously steeped in sterile vaseline and picric acid, are spread over the skin about the wound, covering every part of the skin but not any of the

wound or granulating surface. The hair in the neighborhood of the wound is kept shaved close to the skin.

The fact that we are aiming to irrigate and not to drain must be kept in mind when we are placing the tubes; they should be placed so as to take full advantage of gravity when irrigating. If they are pushed up into the wound with the outer end extending down, it will be much more difficult to get fluid through to the wound and as much of the fluid as fills the tube will never reach the wound but drain immediately after the pressure is removed. Dakin's solution to retain its efficiency must be protected from light and air, and under no condition should it be warmed. If the free chlorine contained is the active principle of the solution, exposure to air or light or heating the solution will liberate this and therefore impair the efficiency of the treatment.

At the time of the dressing, a thorough irrigation of the wound is advisable both to determine that the tubes are open and working properly and to remove any débris that may have collected. If the surface of the wound is not clean, it should be cleaned with a dry sponge. To keep the tubes from slipping out of the wound they are fastened to the skin with adhesive tape, and sponges wet with the solution are placed loosely over them on the surface of the wound. Dakin's solution dissolves fibrin very readily, thus clearing away and carrying off the fibrin in the pus that might otherwise be deposited. But it also dissolves the fibrin of a blood clot and thus, if a large vessel is ruptured in the wound, treatment with Dakin's solution may be the cause of a secondary hemorrhage.

On every other day an inspection is made of the bacterial content both as to number and variety. No irrigation is permitted for at least two hours preceding this dressing. With a sterile platinum spatula a bit of material is taken from the most vicious part of the wound or, if the wound looks uniformly good, specimens are taken from several points. The spatula must be sterilized each time just before it is applied to the wound. The material is spread in a thin film over a glass slide and allowed to dry. The film should not be too thick, otherwise it may be too dense for examination. In the laboratory this film is stained with methylene blue and examined with an oil-immersion lens. A certain amount of experience will accustom the examiner to choose fields of a certain density as standard. These fields should have the pus cells arranged in a single layer and uniformly covering the whole field. The number and variety of organisms are noted. The noting of large and small bacilli, staphylococci and streptococci, is a sufficient distinction as to variety. In counting, no attention is paid to variety but the organisms are counted as a whole. If there are a hundred or more germs in the field, a second and third field are examined; and, if these, too, show great numbers, the count is said to be "infinity," and no further examination is necessary. If, however, there are only five or

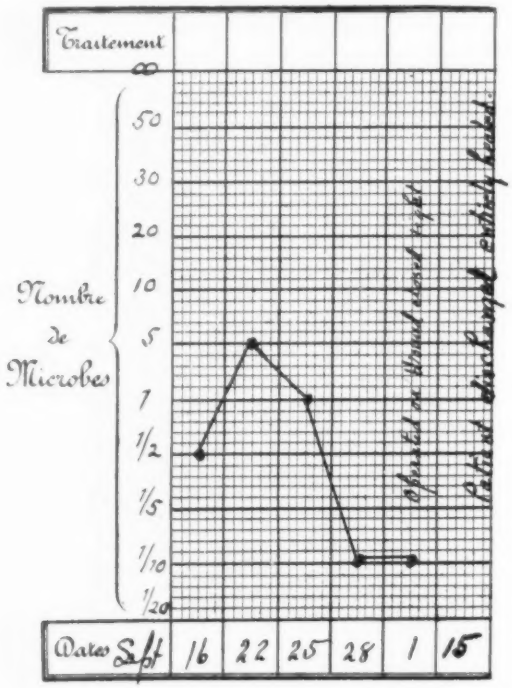


CHART II.

COMPOUND FRACTURE OF THE MIDDLE OF THE LEFT HUMERUS.

**In twelve days the bacterial count was brought to one organism in ten fields. After four days the edges of the wound were freshened and stitched. On October 15, the patient was discharged with only a simple fracture.**

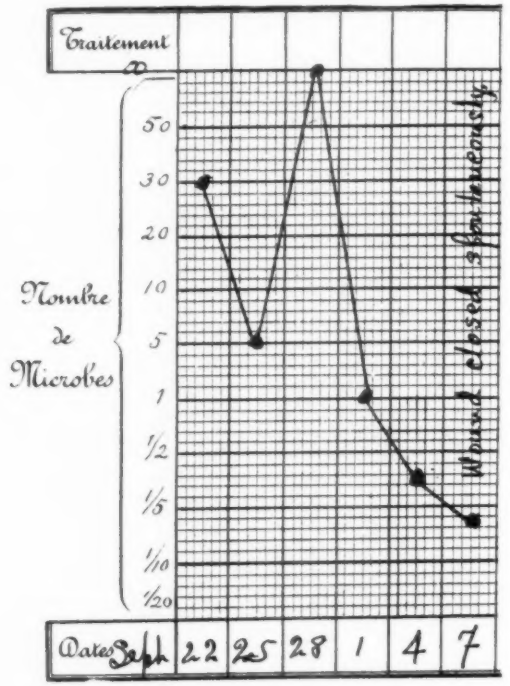


CHART III.

COMPOUND FRACTURE OF THE LEFT SUPERIOR MAXILLARY BONE.

**This wound, after it had been cleaned, closed spontaneously and healed without stitching.**

ten organisms in the first field, then five other fields in different parts of the slide are examined, the total count of all the fields is averaged and this average taken as the count. If the bacterial content is still poorer, perhaps only one or two to a field, at least 10 fields are examined, and when the count is lower than this, 20 or more fields are searched. It often occurs in this latter phase that 10 or 15 fields may be examined without a single organism being noted, and then in the next field a phagocyte will be found which has engorged a score or more of organisms. These phagocyted bacteria are counted or estimated and averaged in the same manner as though the organisms had been scattered extracellularly. The results of these examinations are charted on sheets similar to temperature charts and always kept at hand for the surgeon's inspection. These observations are the index to much valuable information. First, they tell the nature and extent of the infection and whether it is spreading or receding. Later, as parts of the wound clear up, the counts tell which part is not receiving its regular irrigation. If a corner of the wound is blocked off by a gauze packing, the bacterial content of this part will be much higher than that of other parts receiving their regular irrigation. In general, it has been observed that the count drops from infinity to 10 or 20 per field within the first 24 or 48 hours if the irrigation is satisfactory; but if this drop is not observed, a thorough inspection of the technique should be made at once. If this shows no fault, then the wound should be examined for an unexplored and unirrigated pocket. Second, this observation enables one to discover a reinfection before it has become extensive. A sudden rise of the count or the discovery of new organisms indicates a reinfection either from the evacuation of a pocket of pus or from an outside source. Immediate re-establishment of the full irrigation may control this inoculation in a few hours. When the count averages one organism or less to five oil-immersion fields for a continuous space of six days, the wound is considered bacteriologically clean. A certain percentage of such wounds have shown themselves to be absolutely sterile on culture. When surgical repair work is indicated, it can be done at this time as safely and with as much ease and security as though the wound were fresh. The previous infection can be completely ignored.

The great advantages of the Carrel method of treating infected wounds may be summed up as follows: It successfully checks the extension of the infection, causes an almost immediate drop of temperature and brings comfort to the patient. Pus is eliminated and dressing simplified. An early opportunity is given for surgical repair work. The production of cicatricial tissue is minimized and the interference with function of the part is less after the recovery of the patient. Finally, it is a scientific and intelligent method of observing the process of healing in wounds and determining the efficacy of treatment.

# PLACENTAL TRANSMISSION: TOTAL CREATININE IN PLASMA, WHOLE BLOOD AND CORPUSCLES OF MOTHER AND FETUS.

## (ADDITIONAL ANALYSES BY A NEW METHOD.)

By E. D. Plass.

*(From the Department of Obstetrics, Johns Hopkins University and Hospital.)*

In a recent study of the placental transmission of creatinine and creatine, it was found that the maternal and fetal plasmas or sera have the same concentrations of these two substances, but that the whole bloods show the same definite relationship only in the preformed creatinine, whereas the total creatinine and subtracted creatine values are higher in the fetal bloods.[1] It was suggested that the values obtained for total creatinine in whole blood by Folin's method were probably too high. Subsequent observations have indicated that this criticism is valid and by the use of the acetic acid precipitation method, lately suggested, consistently lower results were obtained.[2] The higher values found when the Folin procedure is employed are probably explained by the presence in the blood of some substance, not creatine, which when heated with picric acid, produces a compound which gives a color development on alkalinization similar to that produced by creatinine.

In continuing the previous work, a number of determinations of total creatinine were made on plasmas and whole bloods by means of the new procedure. The preformed creatinine was not determined because the previous analyses had indicated that if the blood is unhemolysed the two samples give approximately the same results. In view of the reported lower creatine content of fetal tissues [3, 4, 5] it seemed probable that the blood cells would show a comparable difference, but such was not the case.

The specimens were collected as in the previous experiments. The hematocrit values were determined by direct centrifugalization of the undiluted whole blood. A known quantity of whole blood or plasma was precipitated by heat and .01 N acetic acid and the filtrate cleared with a suspension of aluminium hydroxide. The second filtrate was evaporated to 5 c. c. on a water-bath and taken up with hot water, the total volume being kept at approximately 10 c. c. One cubic centimeter of 5N HCl was added and the solution heated on a water-bath for from 3 to 4 hours, after which it was neutralized with 20 per cent NaOH and 20 c. c. of 1.2 per cent picric acid were added. Next there were introduced 1.5 c. c. of 10 per cent NaOH to develop the color, and after 10 minutes the solution was diluted to 50 or 100 c. c. and the color compared with that produced by a known amount of creatinine treated similarly.

The results in a series of 12 sets of plasmas or sera are essentially the same as those previously obtained by the use of

Folin's method, and the agreement between the maternal and fetal samples confirms the view that the placental transmission of the creatinine bodies is by simple diffusion. The actual findings are recorded in Table I; a statement as to the presence or absence of hemolysis is included to emphasize the fact that the presence of hemoglobin does not materially affect the results obtained by the use of this method.

TABLE NO. I.

TOTAL CREATININE IN MATERNAL AND FETAL PLASMAS.

(Mg. creatinine in 100 c. c.)

| Source. | Maternal. | | Fetal. | |
|---|---|---|---|---|
| Case No. | Hemoglobin. | Total Creatinine. | Hemoglobin. | Total Creatinine. |
| 26 | 0 | 1.74 | 0 | 2.04 |
| 29 | 0 | 1.65 | + | 1.85 |
| 30 | 0 | 2.02 | + + | 2.31 |
| 31 | 0 | 1.69 | + | 1.72 |
| 32 | + | 1.90 | + + | 1.90 |
| 33 | 0 | 1.80 | + | 1.79 |
| 34 | + + + | 2.06 | + + | 1.75 |
| 35 | + + + | 1.62 | + + | 1.63 |
| 36 | + + | 1.39 | + + | 1.27 |
| 40 | + + + | 1.16 | + + + | 1.15 |
| 41 | 0 | 1.19 | + | 1.40 |
| 43 | + + + | 2.12 | + + + | 2.00 |
| Average | | 1.70 | | 1.73 |

Ten sets of maternal and fetal whole bloods were analyzed, and in seven the plasmas were also available. The results are tabulated in Table II, as are also the calculated total creatinine contents of the corpuscles expressed in milligrams per 100 c. c. Here, again, we find the same concentration in the plasmas of both mother and child.

The amounts of total creatinine in the whole blood and corpuscles, however, do not show any such parallelisms. The quantities in the maternal whole blood are higher in three instances, in the fetal in six, and in one (No. 36) there is no difference; the average contents are quite comparable. The calculated corpuscular content is higher in the maternal sample in five cases, in the fetal in three, and in two only a slight difference is noted. The age, color and parity of the mothers, the sex and weight of the children, the duration of labor and various clinical details were tabulated, but gave no suggestion regarding the reason for the observed variations. Until some explanation for these differences of storage in the blood cells is proposed, no attempt can be made to correlate the findings here recorded.

As the preformed creatinine seems to be present in small and rather uniform quantities in both plasma and corpuscles, the relatively large amounts of total creatinine are apparently due to a storage of creatine. Many of the tissues of the body contain creatine, but very little creatinine, and it is not surprising that the blood cells can store small quantities of the former substance. In the non-pregnant state this storage is apparently smaller (6.2-6.5 mg. per 100 c. c.) than during pregnancy, whereas in the male a single determination showed a still lower concentration (4.9 mg. per 100 c. c.). In the tabulated series in only one instance (Case No. 41) is there no evidence of a considerably increased storage; whereas the average figures are nearly double the normal. There are no available figures bearing upon the relative concentration of creatine in the muscle and other tissues in the non-pregnant state and during gestation, so it cannot be said whether this phenomenon of creatine storage is general or affects only the blood cells.

TABLE NO. II.

TOTAL CREATININE IN WHOLE BLOOD, PLASMA AND CORPUSCLES.

(Mg. creatinine in 100 c. c.)

| Source. | Maternal. | | | | Fetal. | | | |
|---|---|---|---|---|---|---|---|---|
| Case No. | Hemato-crit. | Whole Blood. | Plasma. | Corpuscles. | Hemato-crit. | Whole Blood. | Plasma. | Corpuscles. |
| 34 | 31 | 6.16 | 2.06 | 15.29 | 34 | 5.03 | 1.75 | 11.38 |
| 35 | 37 | 4.76 | 1.62 | 10.11 | 52 | 5.40 | 1.63 | 8.88 |
| 36 | 32 | 5.33 | 1.39 | 13.69 | 44 | 5.37 | 1.27 | 10.59 |
| 37 | 31 | 4.44 | 1.70* | 10.55 | 30 | 5.67 | 1.70* | 14.93 |
| 38 | 28 | 5.41 | 1.70* | 14.96 | 29 | 5.67 | 1.70* | 11.14 |
| 39 | 34* | 4.91 | 1.70* | 11.15 | 40* | 4.47 | 1.70* | 8.63 |
| 40 | 33 | 3.40 | 1.16 | 7.94 | 42 | 4.42 | 1.15 | 8.93 |
| 41 | 39 | 3.06 | 1.19 | 5.99 | 44 | 3.56 | 1.40 | 6.32 |
| 43 | 37 | 5.55 | 2.12 | 11.38 | 42 | 6.25 | 2.00 | 12.12 |
| 44 | 39 | 3.65 | 1.35 | 7.26 | 43 | 5.92 | 1.35† | 11.98 |

* Actual determinations not made—average values taken.
† Not determined—maternal value used.

## SUMMARY AND CONCLUSIONS.

Previous work on the total creatinine content of maternal and fetal plasma and whole blood was repeated, a different analytical procedure being employed. No definite relationship has been shown to exist between the maternal and fetal whole bloods in a given case, but the plasma values in both series of experiments agree closely, indicating a direct diffusion of the creatinine bodies through the placenta.

In the parturient woman and in the new-born child there is usually an increased ability of the red blood cells to store creatine. In spite of the reported lower creatine content of fetal tissues, the maternal corpuscles do not always show a higher creatine content than the fetal cells.

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# NOTES ON NEW BOOKS.

*Hand-Book of Venesection.* By WALTON F. DUTTON, M. D. Cloth, $2.50. (*Philadelphia: F. A. Davis & Co., 1916.*)

This small volume, of about two hundred pages, is an attempt to present to the profession an epitome of the indications for venesection, with a broad discussion of the physiological effects that may be obtained in each instance.

The first forty pages are devoted to a fascinating history of Bloodletting, taken from a paper by Fielding H. Garrison, read before the Medical History Club of Washington, D. C., on December 30, 1911.

The remainder of the book consists of a category of diseases which may be treated by venesection or which have been so treated in the past. The discussion of the physiology of disease is largely good, though one is struck by the undue emphasis placed by the author upon vasomotor disturbances of the cerebral circulation. Such disturbances, the author claims, are the cause of epilepsy which should be treated by venesection. A chapter is devoted to hypertonia vasorum cerebri, which seems to be an entirely hypothetical condition, but which is, nevertheless, to be treated by venesection.

The author advocates, with good physiological reasoning, the use of venesection under certain circumstances in aneurism, emphysema, angina pectoris, cerebral hemorrhage, asphyxia, arteriosclerosis, pneumonia, eclampsia, gout with uremia, pulmonary edema and congestion, uremia and various cardiac conditions. He places proper emphasis upon dilatation of the right heart, cyanosis, and venous engorgement as being of primary importance for the indication of venesection.

The book is, however, that of an enthusiast, and one is startled by the list of conditions which are to be treated, under some circumstances, by venesection—rheumatism, syphilis, obesity, migraine, hypertonia vasorum cerebri, typhoid fever, epilepsy, psychopathic states, hypertension, etc.

The technique of venesection and of transfusion as described by the author belongs to the surgical procedures in vogue a few years ago, and no description is given of the new and much more readily applicable methods of venesection and transfusion through needles.

The book is interesting but somewhat optimistic as to therapeusis by venesection. J. T. K., JR.

*The American Year-Book of Anesthesia and Analgesia.* Edited by F. H. MCMECHAN, A. M., M. D. Cloth, $4.00. (*New York: Surgery Publishing Co., 1915.*)

The American Year-Book of Anesthesia and Analgesia is made up of a collection of articles by prominent authorities on this subject. The table of contents shows an impressive list of contributors from all over the States and from England as well, with papers covering the subject from all aspects, from the deeply scientific to the extremely practical. It will prove of great interest to anesthetists rather than of actual value to the student who is giving his first anesthetic. The papers are slightly contradictory on some points which goes to show that there is much about the subject that has yet to be brought down to indisputable fact. It is interesting to find one of the articles by a lay woman whose point of view coincides with that of most patients and should be respected and born in mind constantly. Those who read this year-book will anticipate with eagerness next year's volume.

*Diseases of the Nose and Throat.* By ALGERNON COOLIDGE, A. B., M. D. Cloth, $1.50. (*Philadelphia: W. B. Saunders Co., 1915.*)

This little book fills its purpose admirably. As stated in the preface, it purports to be only a manual and does not attempt any complete discussion of the subject.

The words are well chosen, the sentences are short and concise. The subject matter is, on the whole, quite accurate, though certain phases do not receive the importance they deserve. For instance, the relation of sinus infection to asthma is not altogether clear. The indications for tonsillectomy are clearly expressed and complete. There is an excellent table treating of the differential diagnosis between syphilis, tuberculosis and malignant neoplasms.

The illustrations are all schematic, but give the student a very good idea of anatomical relations. The author has very wisely omitted all illustrations of instruments and discussions of various methods of operations. This is quite a departure from the ordinary book on this subject and is a great relief. A concise table of contents and index add greatly to the value of the book from the student's point of view. H. R. S.

*Diagnostic Methods: Chemical, Bacteriological and Microscopical.* By RALPH W. WEBSTER, M. D. 5th Edition. Cloth, $4.50. (*Philadelphia: P. Blakiston, Son and Co., 1916.*)

The fact that this book has gone through five successive editions since 1909 is in itself testimonial enough as to its contents and its value in practical clinical medicine. The author is to be congratulated for the zeal which prompts him to keep abreast with all valuable advances in the line of clinical laboratory methods to such an extent that a new edition can be published in each of four successive years. Despite the curtailment of scientific investigation as one result of the war, a sufficient amount of new material has been brought together in this present volume to give the book 725 pages of text in which can be found practically all acceptable methods for the analysis of the ordinary laboratory specimens. The arrangement of the subject matter is orderly and logical, the descriptions of methods are uniformly clear and singularly free from error. Included in the subject matter are numerous references of value to the original literature. The author is to be congratulated upon his obviously successful effort to keep this book foremost among those dealing with the subject of clinical microscopy. S. R. M.

*The Healthy Girl.* By MRS. J. CUNNING, M. B., and A. CAMPBELL, B. A. Cloth, $1.75. (*Oxford University Press, London, 1916.*)

Written by authors possessing a good insight and understanding of the growing mind and body, this book is intended for the growing girl and those adults who have her welfare in charge. The greater part of it, however, is better adapted to the adult than to the growing girl herself. A concise, common sense and very practical survey of the anatomy and physiology of the human body in their relation to health and disease is given in a simple and easily understood manner. A number of illustrations help to elucidate the text. The weak points in the book are to be found in the attempts to give medical advice. The common diseases are briefly described and treatment in some cases is suggested: "use mufflers for sore throats, use a hot-water bottle for appendicitis and in general for any illness where a girl complains of severe pain." "Coffee is a laxative." "In scarlet fever swab the throat every few hours with 1-30 carbolic." The necessity of a doctor's advice in all illness is, however, repeatedly emphasized. The personal hygiene of the growing girl is well handled and the question of regulation of exercise, work, bathing, sleep and fresh air is ably discussed with helpful suggestions as to the planning of the daily routine.

M. D. B.

*Nervous Asthma. Its Pathology and Treatment.* By J. B. BERKHART, M. D. Price, 2 shillings and 6 pence. (*Oxford University Press, London, 1916.*)

According to the author two distinct types of asthma are usually grouped under the name bronchial, spasmodic or nervous asthma. Both types, he believes, are the consequence of rickets with its resulting crippling of the thorax and arrested lung development. In the one form, which he designates as actual bronchial asthma, the dyspnea arises from transient obstacles in the bronchial tree—obstacles resulting from the "presence of a tenacious pathological product associated with a defect in the power of expectoration."

In the second form, described as true nervous asthma, no real mechanical obstacle to breathing is supposed to exist, but a neuropathic constitution, with its associated hypersensitiveness to all stimuli, is always present. Furthermore, in all these nervous cases there is a history of childhood inflammatory affections of the air passages and the author believes that these organs, therefore, probably afford a stimulus sufficient to stir up the underlying pathological constitution. Many cases, however, he thinks are pure anxiety neuroses. He lays great stress upon the benefit derived in all cases of nervous asthma from the treatment of the underlying neuropathic constitution by means of proper hygiene, change of surroundings and freedom from solicitous friends. His statements are emphasized by frequent recitals of case reports.

The section of the book dealing with the pathology of the condition is the weakest. The author makes dogmatic statements without attempting to give any evidence. His pathological ideas are unusual and are not presented in a manner which would tend to convert others to his way of thinking.

*Principles and Practice of Obstetrics.* Joseph B. De Lee, M. D. Second edition. Cloth, $8.00. (*Philadelphia: W. B. Saunders Co., 1915.*)

Dr. De Lee's book is enjoying a well deserved popularity and it has undoubtedly proven itself to be a valuable practical work on the subject of obstetrics.

At times the author perhaps shows a tendency to be rather too dogmatic, as, for example, in his discussion of the life history of the corpus luteum where he hardly gives proper consideration to the more generally accepted view in regard to the origin of the lutein cells.

The chapters devoted to the physiology of labor are particularly good. The various pathological conditions which may arise during the course of pregnancy, labor and the puerperium are discussed at length, but more particularly from their practical aspects. Although the scientific side has not been neglected, it does not seem that the author has drawn sufficient attention to the numerous unsolved problems existing in scientific obstetrics, which will offer a fruitful field for investigation for a long time to come.

In the section on operative obstetrics, the author describes with great clearness and detail the various steps employed in the operative procedures employed in modern obstetrics.

The book is profusely illustrated with particularly clear and instructive illustrations and diagrams.

In the bibliography, it seems that it would have been an advantage to give with uniformity the titles of the various articles referred to.

Dr. De Lee's book should continue to prove itself to be of great practical assistance to the student and practitioner. K. W.

*The Difficulties and Emergencies of Obstetric Practice.* Comyns Berkley, M. D., and Victor Bonney, M. D. Second Edition. Cloth, $7.50. (*Philadelphia: P. Blakiston's Son & Co., 1915.*)

In the second edition of this work, the authors follow along the lines laid down in the first edition and discuss only the abnormalities encountered in obstetric practice. That there is a distinct field for such a work is proven by the speedy exhaustion of the first edition.

The various obstetrical complications are discussed in much greater detail than is possible in the ordinary text-book on obstetrics.

The section describing the various obstetrical operations is excellent. The book contains numerous illustrations which are unusually clear and instructive, a number of them being entirely new.

The work should continue to prove of value to the student and to the practitioner. K. W.

# AN APPEAL TO THE MEDICAL PROFESSION OF THE UNITED STATES.

The Surgeon General's Office has appealed to the medical press of this country for aid in securing the quota of physicians necessary for the care of the great army now in course of organization.

The Medical Departments of the Government are responsible for the examination of the recruits, the hygiene of camps and the care of the sick and wounded. The Surgeons General have not as yet been given full authority and the means to meet this responsibility.

The President and Congress can give the Surgeons General full authority and ample means, but except by the draft neither the President nor Congress is able to give them a sufficient number of men from the medical profession, as it is a volunteer service. Consequently, if the Medical Departments are furnished with the authority and the means, they will still be unable to do their work unless the medical profession of the country, and particularly the younger men, respond more freely than they have done up to this time.

In the army hospitals and first aid work abroad, in the vast concentration camps so soon to be organized in this country, and in every branch of the naval service there is an urgent demand for each physician who can and will offer his services. In the work our country has pledged itself to do, the need for doctors is imperative. Estimates give the figure of 20,000 physicians as the minimum number necessary for this work. Only about 6000 are at present enrolled. These figures speak for themselves.

Commissions in the Medical Reserve Corps are accorded on the basis of First Lieutenant, Captain and Major, with respective salaries of $2000, $2400 and $3000 a year. Applicants may apply directly to the various examining boards throughout the different states and complete all preliminary arrangements without reference to the Surgeon General's Office. The completed papers should be forwarded directly to the Surgeon General by the president of the examining board. A complete set of papers must contain the physical examination, report of the examiner as to mental, moral and physical qualifications, a personal history form filled out by the applicant and sworn to before a notary, and a certificate of state registration (except where this year's graduates have not had time to take their State Board examination). Two letters should also be sent certifying as to citizenship and moral qualifications, and, if of alien birth, a certificate of naturalization.

Further information may be obtained from the State and County Committees of National Defense, or directly from Surgeon General W. C. Gorgas, of the Army, or Surgeon General W. C. Braisted, of the Navy, Washington, D. C.